One-Electron Oxidation of 6-Substituted Benzo[a]pyrenes by Manganic Acetate. A Model for Metabolic Activation

Paolo Cremonesi, Ercole L. Cavalieri,* and Eleanor G. Rogan

Eppley Institute for Research in Cancer and Department of Pharmaceutical Sciences, University of Nebraska Medical Center, 42nd St. and Dewey Ave., Omaha, Nebraska 68105-1065

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Radical cations of benzo[a]pyrene (BP) and 6-substituted derivatives were generated by one-electron oxidation with 2 equiv of $Mn(OAc)_3 \cdot 2H_2O$. Some of the properties of these radical cations were investigated by nucleophilic trapping with acetate ion. BP produced predominantly 6-OAcBP and small amounts of BP 1,6-, 3,6-, and 6,12-dione. 6-FBP yielded 6-OAcBP, a mixture of 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP, and BP diones. In the case of 6-ClBP and 6-BrBP the major products obtained were a mixture of the 1-OAc and 3-OAc derivatives, and BP diones, while substantial starting material remained unreacted. 6-CH_3BP afforded mostly 6-$OAcCH_2$BP, a mixture of 1-OAc and 3-OAc derivatives of 6-CH_3BP, and a mixture of 1-OAc and 3-OAc derivatives of 6-$OAcCH_2$BP. These results indicate that nucleophilic substitution of $BP^{\bullet+}$ and 6-$FBP^{\bullet+}$ occurs exclusively at C-6. For 6-$ClBP^{\bullet+}$ and 6-$BrBP^{\bullet+}$ substitution at C-1 and C-3, which are the positions of second highest charge density in their radical cations after C-6, compete successfully for nucleophilic substitution. For 6-$CH_3BP^{\bullet+}$ charge localization at C-6 activates the methyl group rendering it the most reactive toward nucleophilic attack. Competitive acetoxylation of 6-$CH_3BP^{\bullet+}$ also occurs to a minor extent at C-1 and C-3. These mechanistic studies have been useful in clarifying some aspects of the metabolism of BP and its halogeno derivatives by cytochrome P-450 and peroxidases. Furthermore, this chemistry can provide some guidance in understanding the mechanism of tumor initiation by these compounds.

Introduction

Covalent binding of chemicals to cellular macromolecules, DNA, RNA, and protein, is the first critical step in the complex process leading to tumor formation.[1,2] The critical intermediates responsible for reacting with cellular macromolecules have a common, unifying feature, their electrophilic character.[1,2] Polycyclic aromatic hydrocarbons (PAH), as most other chemicals, require metabolic activation to produce the electrophilic species responsible for reacting with cellular nucleophiles.

Because the covalent bond between PAH and macromolecules is critical in triggering the tumor process, the biological chemistry of PAH must fit with their carcinogenic activity. This means that the carcinogenicity of PAH must be coherent with certain common chemical properties of these compounds and the catalytic properties of the enzymes responsible for their activation. This basic concept represents the essence for unraveling the complexity of the mechanisms of carcinogenesis by PAH.

Metabolic activation of PAH can be understood in terms of two main pathways: one-electron oxidation to yield reactive intermediate radical cations[3-5] and monooxygenation to produce bay-region diol epoxides.[6-8] Thus, activation of PAH can proceed through one of these pathways or a combination of both. Numerous experimental results concerning activation of PAH to bay-region diol epoxides have led the scientific community to think that this mechanism is almost exclusive in PAH carcinogenesis.[6-8] However, the results of many carcinogenicity experiments with the most potent PAH, benzo[a]pyrene (BP), 7,12-dimethylbenz[a]anthracene, and 3-methylcholanthrene, do not support an important role for the diol epoxide pathway.[3,9,10]

The reason we have postulated that one-electron oxidation plays an important role in the activation of PAH derives from certain characteristics of the radical cation chemistry of these compounds common to the most potent PAH: (1) a relatively low ionization potential (IP), which allows easy metabolic removal of one electron with formation of a radical cation,[11,12] (2) charge localization in the PAH radical cation that renders this intermediate specifically and efficiently reactive toward nucleophiles,[3,13-15] and (3) optimal spatial configuration that presumably facilitates formation of appropriate physical complexes with cellular macromolecules and thus favors metabolic activation by one-electron oxidation.[16-18]

Mammalian peroxidases, including prostaglandin H synthase,[19-22] and cytochrome P-450[23-30] catalyze one-

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electron oxidation. This mechanism is involved not only in the formation of metabolites but also in binding of BP to DNA, as we have recently demonstrated for horseradish peroxidase[31] and cytochrome P-450.[10,32]

Study of the radical cation chemistry of BP and other PAH is essential to understand the mechanism of biological activation of these compounds. Radical cations of PAH have been generated electrochemically[33] and chemically with Fe(III),[34-37] iodine,[13,14,34,36,38-40] and $Mn(OAc)_3$.[3,15] Nucleophilic substitution of $BP^{\bullet+}$ proceeds almost exclusively at position 6.[14,36-43]

We have specifically investigated the properties of the radical cations of BP and 6-substituted derivatives generated by one-electron oxidation with $Mn(OAc)_3 \cdot 2H_2O$. The mechanistic studies have been useful in clarifying some aspects of the metabolism of the 6-halogeno derivatives of BP by cytochrome P-450[23] and peroxidases.[44] Furthermore, this chemistry can provide some guidance in understanding the mechanism of tumor initiation by these compounds.[9,45]

Experimental Section

All ^1H NMR spectra were recorded in $CDCl_3$ with $(CH_3)_4Si$ as internal standard on a Varian XL-300 instrument at 300 MHz. Chemical shifts are expressed as ppm. Assignment of ^1H NMR spectra was achieved by applying empirical rules that designate different chemical shifts for protons bound to certain carbon atoms (e.g., α- or β-naphthalenic, *meso*-anthracenic, etc.).[46] Support for the assignments has been obtained by spin–spin decoupling and two-dimensional chemical shift correlation spectroscopy (2D COSY). Mass spectra were recorded on a Kratos MS-50 instrument. HPLC analyses were performed on a Spectra Physics 8100 instrument with UV detection at 254 nm and a computing integrator. Routine analyses of PAH were run on an Altex Ultrasphere ODS 5 μm column using a 60-min gradient from 60% methanol in H_2O to 100% methanol at a flow rate of 1 mL/min. In some cases improved peak separation and resolution were achieved on a YMC AQ-313 ODS 5 μm column (YMC Inc., Morris Plains, NJ) used in combination with an HP 1090 liquid chromatograph equipped with a diode-array detector (Hewlett-Packard, Palo Alto, CA). Yields of reaction products were calculated from peak areas using molar extinction coefficients determined for each compound at 254 nm in methanol. PAH were also analyzed by TLC on silica gel (Eastman Chromagram) in the following solvent systems: hexane, hexane/benzene (7:3 and 1:1), benzene, and CH_2Cl_2/hexane (7:3 and 8:2). Low-pressure liquid chromatography (100–200 psi) was performed using the Michel-Miller high-performance low-pressure liquid chromatography system (Ace Glass, Vineland, NJ) packed with LPS-1 silica gel (Whatman, Clifton, NJ) and a Lab Pump Model RP SY (FMI, Oyster Bay, NY). Hexane/CH_2Cl_2 gradients were used as elutants, and the compounds were monitored at 254 nm.

Electrochemical syntheses were conducted using 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 platinum working electrode, a saturated calomel reference electrode, 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. In addition, the potentiostat was connected to a digital multimeter (Model 8060A, Fluke Mfg. Co., Everett, WA) so that the electrolysis current could be read. Anodic and cathodic peak potentials were measured by cyclic voltammetry.

Cyclic voltammetry experiments were performed with a Bioanalytical Systems Model CV-27 voltammograph (Lafayette, IN) with either a glassy carbon or a platinum working electrode. A three-electrode cell configuration with an Ag/AgCl reference electrode and a platinum wire counter electrode was employed. The supporting electrolyte was 0.5 M $KClO_4$ in DMF dried by passage over an alumina column. Solutions of 1–2 mM PAH in DMF were used. Potential was scanned between 0 and +1.60 V at a 200 mV/s scan rate. Voltammograms were recorded on a Houston Omnigraphic Model 100 X-Y recorder (Houston, TX), and peak potentials were read on a Fluke Model 8060 Multimeter to three decimal places.

Chemicals. Melting points are uncorrected. All solvents used in reactions were dried and distilled under argon. Benzene was dried over Na, AcOH over P_2O_5, THF over $LiAlH_4$ and CCl_4 over KOH.

$Mn(OAc)_3 \cdot 2H_2O$ was synthesized by $KMnO_4$ oxidation of $Mn(OAc)_2 \cdot 4H_2O$ (Alfa Products, Danvers, MA) in boiling glacial AcOH with 80% yield.[47] The compound was used without further purification and assayed by iodometric titration with a potentiometric end-point determination. The purity was 94–98%. Some experiments required anhydrous conditions. For these, $Mn(OAc)_3 \cdot 2H_2O$ was dried for 2 weeks in a drying apparatus (0.7 mmHg, 100 °C). The compound changed from light brown to brownish black. Titration corresponded to $Mn(OAc)_3$ without any water of crystallization. The same titration results were obtained with $Mn(OAc)_3 \cdot 2H_2O$ purchased from Alfa and dried by the same method. This $Mn(OAc)_3$ will be referred to as "anhydrous" in this paper.

BP (Aldrich Chem. Co., Milwaukee, WI) was purified by column chromatography on alumina eluted with hexane/benzene (1:1) and recrystallized from benzene/methanol, mp 176–178 °C. ^1H NMR: δ 7.75–7.85 (m, 2 H, 8-H, 9-H), 7.90 (d, 1 H, 4-H), 7.97 (d, 1 H, 5-H), 7.97 (t, 1 H, 2-H), 8.07 (d, 1 H, 3-H), 8.22 (d, 1 H, 1-H), 8.26 (dd, 1 H, 7-H), 8.29 (d, 1 H, 12-H), 8.47 (s, 1 H, 6-H), 8.99–9.02 (m, 2 H, 10-H, 11-H).

6-CH_3BP was synthesized by the method of Dewhurst and Kitchen[48] and purified as previously described,[49] mp 215–216 °C.

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^{1}H NMR: δ 3.26 (s, 3 H, 6-CH_3), 7.82–7.87 (m, 2 H, 8-H, 9-H), 7.96 (d, 1 H, 4-H), 7.98 (t, 1 H, 2-H), 8.07 (d, 1 H, 3-H), 8.21 (d, 1 H, 1-H), 8.27 (d, 1 H, 12-H), 8.36 (d, 1 H, 5-H), 8.58–8.61 (m, 1 H, 7-H), 9.07 (d, 1 H, 11-H), 9.11–9.14 (m, 1 H, 10-H).

1-OHBP and 3-OHBP were obtained from the National Cancer Institute Chemical Carcinogen Repository.

6-Bromobenzo[a]pyrene. This compound was obtained by reaction of BP (1 mmol) in 50 mL of dried glacial AcOH at 50 °C and $PyHBr_3$ (1.15 mmol), which was added in small portions as a solid over 10 min. The reaction mixture was then heated on a steam bath and stirred. After 1 h it was cooled and filtered, and the yellow precipitate was washed three times with H_2O, redissolved in 50 mL of $CHCl_3$, washed twice with an equal volume of H_2O, and dried over Na_2SO_4. The solvent was evaporated, and 6-BrBP was chromatographed on silica gel with hexane. The yellow product was obtained in 80% yield after recrystallization from acetone/methanol, mp 216–218 °C (lit.[49] mp 223–224 °C). ^{1}H NMR: δ 7.83–7.89 (m, 2 H, 8-H, 9-H), 7.99 (t, 1 H, 2-H), 8.01 (d, 1 H, 4-H), 8.12 (d, 1 H, 3-H), 8.24 (d, 1 H, 1-H), 8.32 (d, 1 H, 12-H), 8.55 (d, 1 H, 5-H), 8.85–8.88 (m, 1 H, 7-H), 9.00 (d, 1 H, 11-H), 9.04 (d, 1 H, 10-H).

6-Fluorobenzo[a]pyrene. This synthetic procedure was designed in our laboratory with the purpose to improve the yield. 6-BrBP (1 mmol) dissolved in 12.5 mL of anhydrous THF under argon was cooled to −55 °C, and n-C_4H_9Li (1.1 mmol, 1.3 M in hexane) was added dropwise with a syringe. The solution turned black, and the mixture was stirred for 45 min. Perchloryl fluoride was then slowly bubbled into the solution through a needle until the solution turned light orange. To avoid oxidative decomposition, the reaction was rapidly quenched by addition of 5 mL of saturated KI. The solution was allowed to come to room temperature, treated with 10 mL of saturated $Na_2S_2O_3$, and extracted three times with an equal volume of benzene. The combined extract was washed with H_2O and dried over Na_2SO_4. Crude product was chromatographed on silica gel with hexane to give 6-FBP in 55% yield and recrystallized from acetone/methanol, mp 167–169 °C (lit.[50] mp 165 °C). ^{1}H NMR: δ 7.82–7.86 (m, 2 H, 8-H, 9-H), 7.91 (d, 1 H, 4-H), 7.96 (t, 1 H, 2-H), 8.04 (d, 1 H, 3-H), 8,19 (d, 1 H, 1-H), 8.21 (d, 1 H, 12-H), 8.22 (d, 1 H, 5-H), 8.54–8.57 (m, 1 H, 7-H), 8.91 (d, 1 H, 11-H), 8.99 (d, 1 H, 10-H).

6-Chlorobenzo[a]pyrene. This compound was obtained by the method of Nonhebel[51] for 9-chloroanthracene. BP (1 mmol) was dissolved in anhydrous CCl_4 (15 mL), and $CuCl_2$ (2.3 mmol) was added as a solid. The mixture was refluxed overnight under N_2 and then cooled to room temperature, and the solid was removed. The crude product was chromatographed on silica gel with hexane to give a yellow solid in 68% yield, which was recrystallized from benzene/methanol, mp 210–212 °C (lit.[52] mp 210 °C), with a purity >99% by HPLC analysis. ^{1}H NMR: δ 7.88–7.94 (m, 2 H, 8-H, 9-H), 8.02 (t, 1 H, 2-H), 8.05 (d, 1 H, 4-H), 8.14 (d, 1 H, 3-H), 8.27 (d, 1 H, 1-H), 8.33 (d, 1 H, 12-H), 8.55 (d, 1 H, 5-H), 8.84–8.88 (m, 1 H, 7-H), 9.04 (d, 1 H, 11-H), 9.08 (d, 1 H, 10-H).

1-Acetoxybenzo[a]pyrene and 3-Acetoxybenzo[a]pyrene. 1-OHBP or 3-OHBP (1 mg) was dissolved in 2 mL of pyridine-/$(Ac)_2O$ (1:1) and stirred for 12 h at room temperature. The solvent was vacuum-distilled, and the solid product obtained was purified by preparative TLC eluted with benzene. Pure 1-OAcBP or 3-OAcBP was obtained in quantitative yield. ^{1}H NMR of 1-OAcBP: δ 2.57 (s, 3 H, 1-OAc), 7.78 (d, 1 H, 2-H), 7.77–7.88 (m, 2 H, 8-H, 9-H), 7.90 (d, 1 H, 4-H), 7.99 (d, 1 H, 5-H), 8.08 (d, 1 H, 3-H), 8.30 (dd, 1 H, 7-H), 8.33 (d, 1 H, 12-H), 8.53 (s, 1 H, 6-H), 9.04 (b d, 1 H, 10-H), 9.09 (d, 1 H, 11-H). ^{1}H NMR of 3-OAcBP: δ 2.54 (s, 3 H, 3-OAc), 7.78 (d, 1 H, 2-H), 7.77–7.88 (m, 2 H, 8-H, 9-H), 7.94 (d, 1 H, 4-H), 8.05 (d, 1 H, 5-H), 8.25 (d, 1 H, 1-H), 8.30 (d, 1 H, 7-H), 8.33 (d, 1 H, 12-H), 8.53 (s, 1 H, 6-H), 9.06 (b d, 2 H, 10-H, 11-H).

1,6-Diacetoxybenzo[a]pyrene and 3,6-Diacetoxybenzo[a]pyrene. A solution of 0.1 M NaOAc in 20 mL of glacial AcOH dried over alumina was preelectrolyzed at −0.500 V for 15 min. $(Ac)_2O$ (15 mL) was added, and the mixture was electrolyzed for an additional 15 min. A mixture of BP 1,6-dione and BP 3,6-dione (6 mg each) was added to the solution. An aliquot was analyzed by cyclic voltammetry to measure the anodic and cathodic peak potentials, which were found to be in the range of 0.2–0.6 V. The cell was then connected at a potential of 1.000 V, and the coulometer was set to detect only cathodic current. No current flowed under these conditions. The potential was then slowly decreased to −0.100 V, and an initial current of 2.4 mA was detected. After 60 min the reaction was virtually complete, and the solution became light yellow and fluorescent. The solvent was removed by vacuum distillation, and the residual solid was dissolved in $CHCl_3$ and applied onto a preparative TLC plate, which was eluted with benzene.

The mixture of diacetoxyBPs was separated as a yellow fluorescent material, which by HPLC produced two close peaks with a relative ratio of 1:1. ^{1}H NMR: δ 2.55 (s, 1.5 H, 3-OAc), 2.58 (s, 1.5 H, 1-OAc), 2.69 (s, 1.5 H, 6-OAc in 3-OAc), 2.70 (s, 1.5 H, 6-OAc in 1-OAc), 7.77 (d, 0.5 H, 2-H in 3-OAc), 7.78 (d, 0.5 H, 2-H in 1-OAc), 7.80–7.88 (m, 2 H, 8-H, 9-H), 7.93 (s, 1 H, 4-H in 1-OAc, 5-H in 1-OAc), 7.98 (s, 1 H, 4-H in 3-OAc, 5-H in 3-OAc), 8.07 (d, 0.5 H, 3-H in 1-OAc), 8.21–8.31 (m, 2.5 H, 1-H in 3-OAc, 7-H, 12-H), 8.98 (d, 0.5 H, 11-H in 3-OAc), 9.03–9.06 (m, 1.5 H, 11-H in 3-OAc, 10-H).

Standard Procedure for PAH Oxidation by Manganic Acetate. All glassware was dried in an oven at 120 °C for 24 h and cooled under dry argon. Solvents were added to reaction flasks through a septum using a syringe, and a positive argon atmosphere was maintained while solids were added. PAH (0.1 mmol) was dissolved in 20 mL of dry AcOH at 40 °C under argon. $Mn(OAc)_3{\cdot}2H_2O$ (0.2 mmol) was added as a solid, and the mixture was stirred for 30 min at 40 °C. The initial brown suspension cleared and became brown-orange in color after completion of the reaction. A standard reaction time of 30 min was adopted for all oxidations described below, although BP reacted completely in 10 min. At the end of the reaction, the mixture was cooled to room temperature, an equal volume of $CHCl_3$ was added, and the mixture was poured into an equal volume of 1 M $Na_2S_2O_3$. The two phases were separated, and the aqueous one was extracted three times with an equal volume of $CHCl_3$. The organic extracts were combined, washed with saturated $NaHCO_3$ and H_2O, and dried over Na_2SO_4. The solvent was evaporated, and the product mixture was analyzed by HPLC. Purification of crude products by chromatography on silica gel yielded four main fractions: unreacted BP, 6-FBP, 6-ClBP or 6-BrBP eluted with hexane, monoacetoxy derivatives with hexane/benzene (7:3); diacetoxy derivatives with benzene; and BP quinones with CH_2Cl_2. In some cases the intermediate fraction from hexane/benzene was further chromatographed by normal-phase low-pressure liquid chromatography (100–200 psi) on silica gel eluted with a gradient of 80% CH_2Cl_2 in hexane to 10% CH_2Cl_2 in hexane. Final products were recrystallized from benzene/methanol or acetone/methanol before analytical characterization. This is referred to as the "standard" method.

Anhydrous Procedure for PAH Oxidation by Manganic Acetate. Since traces of H_2O were still present in AcOH after distillation and water of crystallization was present in $Mn(OAc)_3{\cdot}2H_2O$, a reaction procedure was developed using anhydrous $Mn(OAc)_3$ (see above) with addition of $(Ac)_2O$ under slightly different experimental conditions. $(Ac)_2O$ (2% v/v) was added to AcOH at 60 °C. After 10 min anhydrous $Mn(OAc)_3$ was added, and the mixture was stirred under argon for 20 min. It was then cooled to 40 °C, and the PAH was added as a solid. After stirring for 30 min the reaction was stopped and worked up as described above. To ascertain that PAH do not react with $(Ac)_2O$ under the experimental conditions, BP, AcOH, and $(Ac)_2O$ in the same molar ratio as above were stirred for 2 h at 60 °C. No product other than BP was detected by HPLC, and recovery of BP was 97%. This is referred to as the "anhydrous" method.

Manganic Oxidation of BP. HPLC analysis of the product mixture after 10 and 30 min revealed that no further reaction occurred after 10 min. Purification of the crude product on silica gel yielded 6-OAcBP (90% yield), a mixture of BP 1,6-, 3,6-, and

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6,12-dione (5%), and unreacted BP (5%). 6-OAcBP was recrystallized from benzene/hexane, mp 209–210 °C (lit.[49,53] mp 209–210 °C). Definite assignment of the various protons in the ^{1}H NMR spectrum required the additional technique of multiple quantum filtration.[54] ^{1}H NMR: δ 2.67 (s, 3 H, 6-OAc), 7.80–7.89 (m, 2 H, 8-H, 9-H), 7.97–8.02 (m, 2 H, 4-H, 5-H), 7.99 (t, 1 H, 2-H), 8.10 (d, 1 H, 3-H), 8.24–8.27 (m, 2 H, 1-H, 7-H), 8.31 (d, 1 H, 12-H), 9.03 (d, 1 H, 11-H), 9.08 (m, 1 H, 10-H).

The structures of the three diones were determined by comparison with authentic samples on TLC and in HPLC analysis, as well as by UV and NMR spectroscopy. ^{1}H NMR of BP 1,6-dione: δ 6.74 (d, 1 H, 2-H), 7.61 (m, 1 H, 8-H), 7.71 (d, 1 H, 3-H), 7.77 (m, 1 H, 9-H), 7.86 (d, 1 H, 4-H), 8.29 (d, 1 H, 10-H), 8.43 (dd, 1 H, 7-H), 8.50 (d, 1 H, 11-H), 8.59 (d, 1 H, 12-H), 8.62 (d, 1 H, 5-H). ^{1}H NMR of BP 3,6-dione: δ 6.71 (d, 1 H, 2-H), 7.61 (m, 1 H, 8-H), 7.72 (d, 1 H, 1-H), 7.76 (m, 1 H, 9-H), 7.78 (d, 1 H, 12-H), 8.26 (d, 1 H, 10-H), 8.35 (d, 1 H, 11-H), 8.44 (dd, 1 H, 7-H), 8.71 (d, 1 H, 4-H), 8.81 (d, 1 H, 5-H). ^{1}H NMR of BP 6,12-dione: δ 7.57 (s, 1 H, 11-H), 7.69 (m, 1 H, 8-H), 7.78 (m, 1 H, 9-H), 7.85 (t, 1 H, 2-H), 8.17 (d, 1 H, 4-H), 8.23 (d, 1 H, 10-H), 8.23 (d, 1 H, 3-H), 8.45 (dd, 1 H, 7-H), 8.47 (d, 1 H, 5-H), 8.62 (d, 1 H, 1-H).

In another experiment $Mn(OAc)_3 \cdot 2H_2O$ was used in equimolar ratio with BP under the standard conditions. Yields were determined by using HPLC analysis of the crude reaction mixture. 6-OAcBP was formed in 51% yield, BP diones in 9% yield, and 40% of the BP was unreacted.

Manganic Oxidation of 6-FBP. The reaction was complete in 25 min. Purification of the products yielded 6-OAcBP (31%), a mixture of 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP (48%), BP diones (19%), and unreacted 6-FBP (2%). The mixture of 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP exhibited two overlapping peaks on HPLC with a relative ratio of 65:35, respectively. Under standard HPLC conditions, the overlapping peaks corresponding to 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP had retention times very close to that of BP 6,12-dione, and quantitation of the dione was not reliable. However, if the analysis was run at 40 °C on the YMC ODS column, the retention times were shifted so that the two diacetoxyBPs eluted earlier, between BP 1,6-dione and 3,6-dione, leaving the peak of BP 6,12-dione distinct. Thus, by combining both analytic conditions it was possible to identify and quantitate all the products. Diode-array detection provided an excellent way to quantitate products present in trace amounts.

The NMR spectrum of the diacetoxyBP mixture was identical with that of the synthesized mixture (see above) and revealed that 65% corresponded to 1,6-$(OAc)_2$BP and 35% to 3,6-$(OAc)_2$BP. MS showed m/e (relative intensity) 368 (48.3, M), in agreement with an elemental formula $C_{24}H_{16}O_4$, 326 (35.3, M – CH_2CO), 284 (100, M – $2CH_2CO$). To confirm the structures of the diacetoxy derivatives, the mixture was hydrolyzed with $NaOCH_3$. HPLC analysis of the products yielded 67% BP 1,6-dione and 33% BP 3,6-dione, identified by comparison with authentic samples.

In another experiment $Mn(OAc)_3 \cdot 2H_2O$ was used in equimolar ratio with 6-FBP under standard conditions. By HPLC analysis, 6-OAcBP was formed in 57% yield, 1,6-$(OAc)_2$BP plus 3,6-$(OAc)_2$BP, 38%, BP diones, 4%, and 1% of 6-FBP was unreacted.

Manganic Oxidation of 6-ClBP. Aliquots from the reaction mixture taken after 0.5, 1, 2, and 12 h showed that the ratio of the product did not change significantly after 1 h. In fact the reaction was 95% complete in 0.5 h. Isolation of the product yielded a mixture of 1-OAc-6-ClBP and 3-OAc-6-ClBP (33%), BP diones (17%), and unreacted 6-ClBP (50%). The two isomers, 1-OAc-6-ClBP and 3-OAc-6-ClBP, were not separated by HPLC or column chromatography. The mixture was analyzed by NMR spectroscopy. Assignment of the various protons was based on the NMR spectrum of the mixture of the diacetoxyBPs synthesized above. Based on this assignment, the relative ratio of acetoxylation of 6-ClBP at C-1 and C-3 was 3:1. ^{1}H-NMR: δ 2.55 (s, 0.75 H, 3-OAc), 2.57 (s, 2.25 H, 1-OAc), 7.65 (d, 1 H, 2-H), 7.89–7.95 (m, 2 H, 8-H, 9-H), 8.04 (d, 0.75 H, 4-H in 1-OAc), 8.07 (d, 0.25 H, 4-H in 3-OAc), 8.14 (d, 0.75 H, 3-H in 1-OAc), 8.27 (d, 0.25 H, 1-H in 3-OAc), 8.32 (d, 0.25 H, 12-H in 3-OAc), 8.35 (d, 0.75 H, 12-H in 1-OAc), 8.55 (d, 0.75 H, 5-H in 1-OAc), 8.60 (d, 0.25 H, 5-H in 3-OAc), 8.85–8.88 (m, 1 H, 7-H), 9.04 (d, 0.75 H, 10-H in 1-OAc), 9.07–9.11 (m, 1.25 H, 10-H in 3-OAc, 11-H).

Further evidence of the structure of the acetoxy derivatives of 6-ClBP was provided by the mass spectra of the compounds. MS showed m/e (relative intensity) 344 (10.5, M), in agreement with an elemental formula of $C_{22}H_{13}O_2Cl$, 346 (4.1, M + 2), 39% of M, which corresponds to the relative isotopic abundance observed for ^{37}Cl, 302 (100, M – CH_2CO).

Quantitation of the crude reaction mixture by HPLC analysis before workup showed trace amounts of 6-OAcBP (~1%), which was identified by comparison with an authentic sample.

Manganic Oxidation of 6-BrBP. Analogously to 6-ClBP, no change in product distribution was observed after 60 min. The products isolated were a mixture of 1-OAc-6-BrBP and 3-OAc-6-BrBP (29%, which were not separated by column chromatography or HPLC), BP diones (10%), and unreacted 6-BrBP (61%). The mixture of the two acetoxy derivatives was further purified by preparative TLC, and the resulting product was analyzed by ^{1}H NMR: δ 2.55 (s, 0.69 H, 3-OAc), 2.57 (s, 2.31 H, 1-OAc), 7.80 (d, 1 H, 2-H), 7.86–7.93 (m, 2 H, 8-H, 9-H), 8.01 (d, 0.77 H, 4-H in 1-OAc), 8.06 (d, 0.23 H, 4-H in 3-OAc), 8.12 (d, 0.77 H, 3-H in 1-OAc), 8.26 (d, 0.23 H, 1-H in 3-OAc), 8.33 (d, 0.23 H, 12-H in 3-OAc), 8.36 (d, 0.77 H, 12-H in 1-OAc), 8.50 (d, 0.77 H, 5-H in 1-OAc), 8.62 (d, 0.23 H, 5-H in 3-OAc), 8.87–8.91 (m, 1 H, 7-H), 9.02 (d, 0.23 H, 10-H in 3-OAc), 9.04–9.09 (m, 1.77 H, 10-H in 1-OAc, 11-H). The lower field signal of the two singlets was assigned to the acetoxy group at C-1, suggesting that the ratio of the two isomers, 1-OAc-6-BrBP and 3-OAc-6-BrBP, was 77:23.

Further evidence for the structure of the acetoxy derivatives was obtained by mass spectrometry. MS showed m/e (relative intensity) 388 (12.9, M), in agreement with an elemental formula of $C_{22}H_{13}O_2Br$, 390 (12.0, M + 2), 93% of M, which corresponds to the relative isotopic abundance observed for ^{81}Br, 346 (100, M – CH_2CO).

HPLC analysis of the crude reaction mixture before workup did not show any traces of 6-OAcBP.

Manganic Oxidation of 6-CH_3BP. No difference in distribution of reaction products was detected between samples taken at 30 and 60 min. In the oxidation of 6-CH_3BP a complex mixture of products was isolated by column chromatography. Unreacted 6-CH_3BP (9%) eluted with hexane; a mixture of 6-$OAcCH_2$BP, 1-OAc-6-CH_3BP, and 3-OAc-6-CH_3BP with hexane/benzene (7:3); a mixture of 6-$HOCH_2$BP, 1-OAc-6-$OAcCH_2$BP, and 3-OAc-6-$OAcCH_2$BP with benzene, and mono- and dihydroxy derivatives with methanol. The fraction containing monoacetoxy derivatives was further chromatographed on silica gel with hexane/CH_2Cl_2 (3:7) to separate 6-$OAcCH_2$BP in 62% yield from the mixture of 1-OAc-6-CH_3BP and 3-OAc-6-CH_3BP (15%). 6-$OAcCH_2$BP was recrystallized from acetone/methanol to give a yellow product, mp 175.5–176 °C (lit.[55] mp 177–178 °C), identical with an authentic sample. ^{1}H NMR: δ 2.13 (s, 3 H, OAc), 6.27 (s, 2 H, 6-CH_2), 7.84–7.89 (m, 2 H, 8-H, 9-H), 8.00 (t, 1 H, 2-H), 8.02 (d, 1 H, 4-H), 8.12 (d, 1 H, 3-H), 8.26 (d, 1 H, 1-H), 8.34 (d, 1 H, 12-H), 8.39 (d, 1 H, 5-H), 8.57–8.60 (m, 1 H, 7-H), 9.06 (d, 1 H, 11-H), 9.09–9.12 (m, 1 H, 10-H).

The remaining fraction of monoacetoxy derivatives showed only one peak on HPLC, and analysis by MS showed m/e (relative intensity) 324 (16.7, M) in agreement with an elemental formula of $C_{23}H_{16}O_2$, 282 (100, M – CH_2CO). NMR analysis revealed, as anticipated, the presence of the two monoacetoxy isomers with the two methyl singlets at 2.57 and 2.59 ppm in a ratio of 37:63, respectively, and accounting together for 3 H. Based on the spectrum of the diacetoxyBPs and the acetoxy derivative of 6-ClBP and 6-BrBP (see above), the lower field signal at 2.59 ppm was attributed to the 1-OAc derivative. Two additional singlets at 3.25 and 3.26 ppm in 37:63 ratio corresponding to the methyl groups at C-6 accounted together for 3 H. The aromatic region had 7.73–7.76 (2 d separated by ca. 1 Hz and corresponding to the 1 and 3 isomers, 1 H, 2-H), 7.83–7.87 (m, 2 H, 8-H, 9-H), 7.91–8.40 (m, 4 H, ar), 8.58 (m, 1 H, 7-H), 9.02–9.11 (m, 2 H, 10-H,

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11-H). The lower field signal at 3.26 ppm can reasonably be attributed to the 1-OAc derivative. The relative ratio of the two isomers is similar to the two diacetoxy derivatives obtained from manganic oxidation of 6-FBP (see above).

The mixture of diacetoxy derivatives and 6-$HOCH_2$BP was further purified by chromatography on silica gel with CH_2Cl_2/hexane (8:2). The two diacetoxy isomers (8%), which eluted first, were not separated. The NMR spectrum of the mixture of isomers showed two singlets at 2.12 and 2.13 ppm (3 H, together) in the ratio of 34:66, corresponding to the acetoxy group on the methylenic moiety, and two singlets at 2.58 and 2.60 ppm (3 H, together) in the ratio of 34:66, corresponding to the acetoxy group at C-3 and C-1, respectively. Then at δ 6.26 (s, 2 H, 6-CH_2), 7.77–7.80 (2 d separated by ca. 1 Hz and corresponding to the 1 and 3 isomers, 1 H, 2-H), 7.85–7.88 (m, 2 H, 8-H, 9-H), 7.97–8.56 (m, 4 H, ar), 8.56–8.60 (m, 1 H, 7-H), 9.02–9.11 (m, 2 H, 10-H, 11-H). MS showed m/e (relative intensity) 382 (3.4, M) consistent with an elemental formula of $C_{25}H_{18}O_4$, 340 (20.7, M – CH_2CO), 282 (M – [CH_2CO + OAc]).

6-$HOCH_2$BP (3%) had mp 232 °C (lit.[49] mp 231–232 °C) and had NMR and MS spectra identical with those of an authentic sample. The last chromatographic fraction eluted with methanol contained small amounts of unidentified products hydrolyzed by silica gel, which could be acetoxy–hydroxymethyl derivatives.

Manganic Oxidation of 6-OAcBP. The crude reaction mixture was evaporated in vacuum to dryness, and the resulting product dissolved in benzene was purified by preparative TLC eluted with benzene. Three major fractions were isolated: unreacted 6-OAcBP, a mixture of 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP, and BP diones. The products were identified by comparison with authentic samples. During workup and chromatography the diacetoxyBPs were easily hydrolyzed to hydroquinones, which immediately oxidized to quinones. Therefore, quantitation of the reaction products was done by HPLC, which resulted in lesser decomposition of the diacetoxyBPs. The following yields were obtained: 26% diacetoxyBPs, 16% BP diones, and 58% unreacted 6-OAcBP.

In another experiment 6-OAcBP was reacted via the anhydrous procedure. In this case 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP were obtained in 21% yield, BP 1,6-dione and 3,6-dione in 18% yield, and 61% 6-OAcBP remained unreacted.

Manganic Oxidation of 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP. A mixture of diacetoxyBPs obtained from manganic oxidation of 6-FBP was reacted with $Mn(OAc)_3 \cdot 2H_2O$. The mixture was sampled at 5, 30, and 60 min and analyzed by HPLC. The following results were obtained: 32% of the 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP mixture was converted to BP 1,6-dione and 3,6-dione after 5 min, 80% after 30 min, and 82% after 60 min.

In another experiment 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP were reacted via the anhydrous procedure. BP 1,6-dione and 3,6-dione were obtained in 60% yield after 30 min, with 40% of the diacetoxyBPs remaining unreacted.

Manganic Oxidation of PAH in the Presence of 5% H_2O. PAH (0.1 mmol) was dissolved in 20 mL of AcOH at 40 °C under argon. H_2O (1 mL, 55.5 mmol) was added dropwise. $Mn(OAc)_3 \cdot 2H_2O$ (0.2 mmol) was then added as a solid, and the mixture was stirred for 30 min and then worked up as described above. The products were analyzed by HPLC. BP gave 6-OAcBP (82%), BP diones (8%), 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP (1.5%), and unreacted BP (8.5%). 6-FBP gave 6-OAcBP (33.5%), BP diones (32%), and 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP (34.5%). 6-ClBP yielded 1-OAc-6-ClBP and 3-OAc-6-ClBP (13%), BP diones (21%), and unreacted 6-ClBP (66%). 6-BrBP gave 1-OAc-6-BrBP and 3-OAc-6-BrBP (17%), BP diones (17%), and unreacted 6-BrBP (66%). 6-OAcBP yielded 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP (9%), BP 1,6-dione and 3,6-dione (35%), and unreacted 6-OAcBP (56%).

Deuteriodeprotonation of 6-FBP. In a reaction flask cooled to 0 °C was added dropwise D_2O (0.2 mol, 3.6 mL) to $(CF_3CO)_2O$ (0.2 mol, 28 mL) under argon. The CF_3COOD obtained was mixed with an equal volume of $CHCl_3$ at room temperature. 6-FBP (0.37 mmol, 100 mg) was added, and the mixture was stirred at room temperature for 48 h. Then an equal volume of H_2O was added, and the solution was extracted three times with an equal volume of $CHCl_3$. The collected organic extracts were washed with a saturated solution of $NaHCO_3$ and then with H_2O and dried over Na_2SO_4. After evaporation of the solvent the crude product was dissolved in benzene, filtered through an alumina column to yield pure 1,3-dideuteriated 6-FBP in 90% yield, and analyzed. MS m/e (relative intensity): 272 (100, M), 273 (24.6, M + 1). ^1H NMR: δ 7.81–7.85 (m, 2 H, 8-H, 9-H), 7.89 (d, 1 H, 4-H), 7.95 (s, 1 H, 2-H), 8.18 (d, 1 H, 12-H), 8.20 (d, 1 H, 5-H), 8.53–8.56 (m, 1 H, 7-H), 8.88 (d, 1 H, 11-H), 8.94–8.97 (m, 1 H, 10-H).

Bromination of 6-FBP. 6-FBP (1 mmol) was dissolved in glacial AcOH (50 mL) at 50 °C. $PyHBr_3$ (1 mmol) was added in small portions to the stirred solution over 10 min. A yellow precipitate formed, and the mixture was stirred for an additional 30 min. The solution was cooled to room temperature, 50 mL of H_2O was added, and the mixture was extracted three times with an equal volume of $CHCl_3$. The combined organic extracts were washed with saturated $NaHCO_3$ to neutrality and then with H_2O and dried over Na_2SO_4. Removal of the solvent yielded a crude mixture, which was purified by low-pressure column chromatography on silica gel eluted with a gradient of 10% CH_2Cl_2 in hexane to 100% hexane. Pure product was obtained in 78% yield and contained two isomers having very close retention times. NMR analysis of the mixture showed the presence of 1-Br-6-FBP (65%) and 3-Br-6-FBP (35%). ^1H NMR: δ 7.80–7.94 (m, 3.65 H, 2-H, 4-H in 1-Br, 8-H, 9-H), 8.04 (d, 0.35 H, 4-H in 3-Br), 8.18 (d, 0.35 H, 12-H in 3-Br), 8.21–8.34 (3 d, 2 H, 1-H in 3-Br, 3-H in 1-Br, 5-H), 8.57–8.60 (m, 1.65 H, 7-H, 12-H in 1-Br), 8.96 (d, 0.35 H, 10-H in 3-Br), 9.01–9.06 (m, 1.65 H, 11-H, 10-H in 1-Br).

The two isomers were separated by preparative HPLC and analyzed by MS. The first eluted peak had m/e (relative intensity) 348 (100, M) in agreement with an elemental formula of $C_{20}H_{10}BrF$, 350 (97, M + 2), 268 (64.8, M – HBr). The second eluted peak had m/e (relative intensity) 348 (100, M), in agreement with an elemental formula of $C_{20}H_{10}BrF$, 350 (99, M + 2), 268 (69.5, M – HBr).

Results and Discussion

Structure Elucidation of Mixtures of 1-Acetoxy and 3-Acetoxy Derivatives of 6-Substituted BPs. High-resolution NMR spectroscopy allowed assignment of the structures of mixtures of 1-acetoxy and 3-acetoxy derivatives of 6-substituted BPs. These were impossible to separate because of their almost identical physicochemical properties and were also subject sometimes to decomposition, especially on silica gel columns. A mixture of 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP, electrochemically synthesized from a mixture of equal amounts of BP 1,6-dione and 3,6-dione, was analyzed by NMR after purification on HPLC. Comparison of this spectrum to those of the monoacetoxy BP derivatives, namely 1-OAcBP, 3-OAcBP, and 6-OAcBP, allowed us to obtain a plausible assignment of all proton resonances of the mixture. After synthesis of the two diacetoxy derivatives in an unequal molar ratio, the doublet at 8.98 ppm was assigned as 11-H in the 3,6-$(OAc)_2$BP isomer. The lowest field signals, 9.03–9.06 ppm, correspond to the two angular protons 10-H and 11-H of the 3-OAc isomer and 10-H of the 1-isomer. The region between 8.2 and 8.3 ppm integrates for 2.5 protons: 1-H in 3,6-$(OAc)_2$BP and 7-H and 12-H in both isomers. This region is very similar to the one in the spectra of 3-OAcBP and 6-OAcBP. After comparison with the spectra of 1-OAcBP and 6-OAcBP, the doublet at 8.07 ppm can be reasonably assigned to 3-H in 1,6-$(OAc)_2$BP. In the spectrum of 6-OAcBP, the doublets corresponding to 4-H and 5-H overlap to form a distorted triplet in which the midline is by far taller than the two external ones. In the diacetoxyBP mixture, the difference between the inner and outer lines is even stronger, and the two external lines disappear from the spectrum. Both signals corresponding to protons 4-H and 5-H of both isomers resemble singlets in the spectrum of the mixture. Comparison with the same signal in the spectra of 1-OAcBP and 3-OAcBP suggests that the lower field singlet can be attributed to 4-H and 5-H in the 3,6-$(OAc)_2$BP, whereas the higher field signal

Table I. Manganic Oxidation of 6-Substituted Benzo[a]pyrenes under Standard Conditions

hydrocarbon	ionization[a] potential, eV	product yield, % 6-OAcBP	6-OAcCH$_2$BP	1-OAc-6-XBP[b] + 3-OAc-6-XBP	1,6-(OAc)$_2$BP + 3,6-(OAc)$_2$BP	1-OAc-6-OAcCH$_2$BP + 3-OAc-6-OAcCH$_2$BP	BP diones	starting material
BP	7.23	90[c] (89)[d]			0 (3)		5 (4)	5 (4)
6-FBP	7.23	31 (24)		0 (0)	48 (57)		19 (18)	2 (1)
6-ClBP	7.26	0 (traces)		33 (41)	0 (0)		17 (13)	50 (45)
6-BrBP	7.30	0 (0)		29 (40)	0 (0)		10 (9)	61 (51)
6-CH$_3$BP	7.06		62[e]	15		8		9

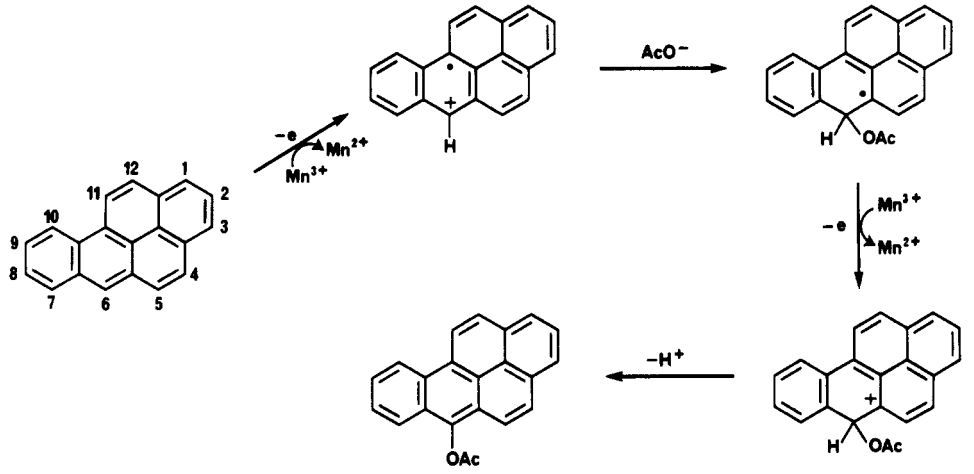
[a] Determined from absorption maximum of the charge-transfer complex of each compound with chloranil.[21] [b] X = F, Cl, Br, CH$_3$. [c] Yield determined by chromatographic isolation of products (85–90% recovery). [d] Yield determined by HPLC. [e] In addition, 3% 6-HOCH$_2$BP was obtained.

Table II. Maganic Oxidation of BP and 6-FBP at Different Molar Ratios of Reactants

PAH	molar ratio Mn^{3+}/PAH	product yield,[a] % 6-OAcBP	1,6-(OAc)$_2$BP + 3,6-(OAc)$_2$BP	BP diones	starting material
BP	2	89	3	4	4
	1	51	0	9	40
6-FBP	2	24	57	18	1
	1	57	38	4	1

[a] Determined by HPLC analysis.

Scheme I. Nucleophilic Substitution in BP$^{\bullet+}$ Generated by Manganic Oxidation of BP



to the same protons in the other isomer. Integration of the multiplet at 7.80–7.88 ppm suggests that these signals correspond to protons 8-H and 9-H of both isomers. Lastly, the two doublets appearing at the highest field correspond to the 2-H proton of both isomers. Homonuclear spin decoupling analysis of the diacetoxyBP mixture established that the signal at 7.77 ppm corresponds to the 2-H of the 3-isomer.

Similar rationales have been applied in elucidation of the structure of mixtures of the 1-monoacetoxy and 3-monoacetoxy derivatives from 6-ClBP, 6-BrBP, and 6-CH$_3$BP. In addition, these criteria were applied to the mixture of 1-OAc-6-OAcCH$_2$BP and 3-OAc-6-OAcCH$_2$BP and the mixture of 1-Br-6-FBP and 3-Br-6-FBP.

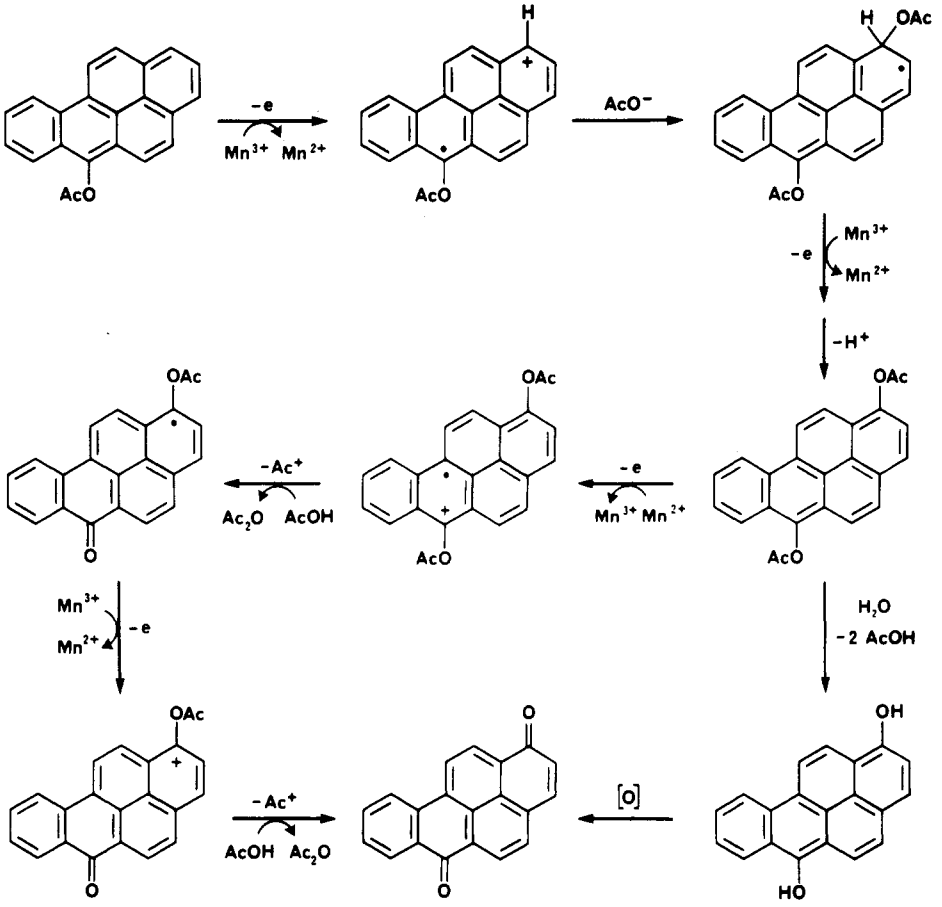
Generation and Nucleophilic Trapping of Radical Cations. BP, 6-FBP, 6-ClBP, 6-BrBP, and 6-CH$_3$BP were studied by reaction with 2 equiv of Mn(OAc)$_3$·2H$_2$O in glacial HOAc. The results obtained under standard conditions are summarized in Table I. The position of substitution reflects the degree of charge localization in the radical cation. All of these compounds have IP that are sufficiently low for an efficient one-electron oxidation by Mn(OAc)$_3$.

BP. In the case of BP, manganic oxidation yields predominantly 6-OAcBP and a small amount of BP diones. Small amounts of diacetoxy derivatives were also detected by HPLC, but they were never isolated because they decompose to BP diones upon column chromatography. Formation of 6-OAcBP involves an initial one-electron oxidation of BP, followed by nucleophilic attack of acetate ion (Scheme I) on the BP$^{\bullet+}$, in which the charge is appreciably localized at C-6. A second one-electron oxidation of the intermediate radical produces an arenium ion, which by loss of a proton yields the acetoxy derivative. This proposed mechanism requires 2 molar equiv of Mn(OAc)$_3$ to complete the reaction. In fact, when the oxidation was conducted with only 1 molar equiv of Mn(OAc)$_3$, 40% of the starting material was left unreacted (Table II).

The formation of small amounts of BP 1,6-dione and 3,6-dione and traces of BP 6,12-dione can derive from competitive nucleophilic attack of H$_2$O at C-6, which is present as H$_2$O of crystallization in Mn(OAc)$_3$ or as residual H$_2$O in glacial HOAc. In fact, by electrochemical oxidation of BP in H$_2$O, Jeftic and Adams[41] obtained formation of BP 1,6-, 3,6-, and 6,12-dione. A second possible path consists of oxidative decomposition and/or acid-catalyzed hydrolysis of the diacetoxy derivatives (Scheme II) produced in trace amount by manganic oxidation of BP (Table I).

To demonstrate that the competitive nucleophilic attack of H$_2$O plays at most a minor role in quinone formation, manganic oxidation of BP was conducted under anhydrous conditions (data not shown). In this case the yield of products is similar to that obtained under standard conditions. When, instead, 5% v/v of H$_2$O was added to the reaction mixture (Table III), formation of quinones only

Scheme II. Nucleophilic Substitution in 6-OAcBP$^{\bullet+}$ Generated by Manganic Oxidation of 6-OAcBP, Followed by Oxidative and/or Hydrolytic Conversion of 1,6-$(OAc)_2$BP to BP 1,6-Dione[a]



[a] Formation of 3,6-$(OAc)_2$BP and BP 3,6-dione not shown.

Table III. Manganic Oxidation of Benzo[a]pyrene and 6-Halogeno Derivatives in the Presence of 5% Water

hydrocarbon	product yield,[a] % 6-OAcBP	1,6-$(OAc)_2$BP + 3,6-$(OAc)_2$BP	1-OAc-6-XBP[b] + 3-OAc-6-XBP	BP diones	starting materials
BP	82	1.5		8	8.5
6-FBP	33.5	34.5	0	32	0
6-ClBP	0	0	13	21	66
6-BrBP	0	0	17	17	66

[a] Yield determined by HPLC. [b] X = F, Cl, Br.

Table IV. Manganic Oxidation of Monoacetoxy and Diacetoxy Benzo[a]pyrenes under Various Experimental Conditions

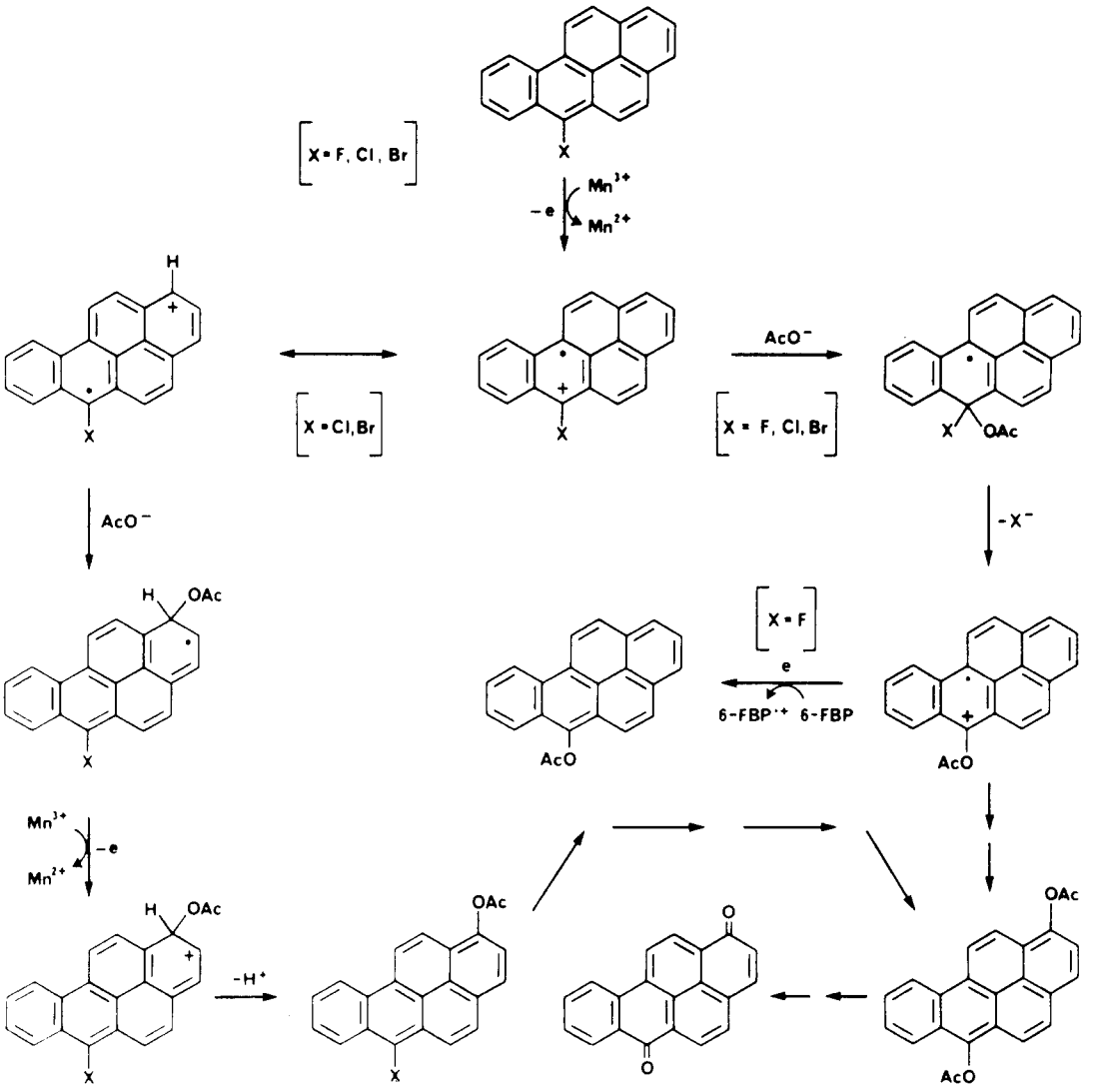
hydrocarbon	oxidation conditions	product yield,[a] % 1,6-$(OAc)_2$BP + 3,6-$(OAc)_2$BP	BP 1,6-dione + BP 3,6-dione	starting material
6-OAcBP				
	standard	26	16[b]	58
	anhydrous	21	18	61
	+ 5% H_2O	9	35	56
1,6-$(OAc)_2$BP + 3,6-$(OAc)_2$BP				
	standard		80	20
	anhydrous		60	40

[a] Determined by HPLC analysis. [b] Trace amounts of BP 6,12-dione were also obtained.

doubled compared to reaction under standard conditions (Table I).

6-OAcBP and DiacetoxyBPs. To demonstrate that oxidative decomposition is involved in the formation of BP diones, manganic oxidation of 6-OAcBP was studied. This reaction produced 1,6-$(OAc)_2$BP, 3,6-$(OAc)_2$BP, BP 1,6-dione, BP 3,6-dione, and a trace amount of BP 6,12-dione (Table IV). The same reaction under anhydrous conditions had similar percentages of quinones produced, suggesting that H_2O of crystallization is not involved in the formation of quinones. However, when 5% H_2O was added to the reaction mixture, a sharp increase in the amount of quinones was observed (Table IV). Reaction of 6-OAcBP with 6 equiv of $Mn(OAc)_3 \cdot 2H_2O$ quantitatively

Scheme III. Nucleophilic Substitution in 6-FBP$^{\bullet+}$, 6-ClBP$^{\bullet+}$, and 6-BrBP$^{\bullet+}$ Generated by Manganic Oxidation of 6-FBP, 6-ClBP, and 6-BrBP[a]



[a] Formation of 3-OAcBP, 3,6-$(OAc)_2$BP and BP 3,6-dione not shown.

yielded BP diones (data not shown). Manganic oxidation of a mixture of 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP efficiently produced BP diones (Table IV).

Formation of diacetoxyBPs from 6-OAcBP occurs by the mechanism proposed in Scheme II. The results obtained by manganic oxidation of 6-OAcBP and diacetoxyBPs (Table IV) suggest that the quinones are formed from oxidation of diacetoxyBPs by two different mechanisms, oxidative decomposition and acid-catalyzed hydrolysis (Scheme II). The mechanism suggested for oxidative conversion to quinones involves one-electron oxidation with loss of an acylium ion followed by a second one-electron oxidation and loss of a second acylium ion (Scheme II). Two combined effects could be responsible for the unusual loss of acylium ion: (1) presumable assistance by the solvent HOAc to form Ac_2O and (2) complementarity of the two acetoxy groups at the positions 1,6 and 3,6 (not shown). In fact, this mechanism is perhaps also operative in the formation of 2-methoxy-1,4-naphthoquinone in the manganic oxidation of 2-methoxynaphthalene, although the intermediate 2-methoxy-1,4-diacetoxynaphthalene was not observed.[56]

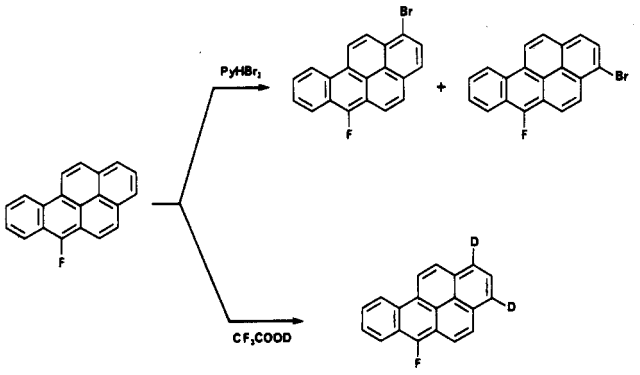
(56) Andrulis, P. J., Jr.; Dewar, M. J. S. *J. Am. Chem. Soc.* **1966**, *88*, 5483–5485.

The second proposed path to formation of quinones involves hydrolysis of the two acetoxy groups, followed by oxidation of the dihydroxyBP in the air to yield quinones.

6-FBP. Manganic oxidation of 6-FBP afforded 6-OAcBP, 1,6-$(OAc)_2$BP, 3,6-$(OAc)_2$BP, and BP diones (Table I). In this case, the reaction was complete when 6-FBP consumed one molar equivalent of $Mn(OAc)_3$ (Table II). This leads us to suggest that formation of acetoxy derivatives occurs by an initial nucleophilic attack of acetate ion at C-6 in the 6-FBP$^{\bullet+}$, followed by loss of a fluoride ion to generate 6-OAcBP$^{\bullet+}$, which yields 6-OAcBP by a final one-electron reduction by 6-FBP to form its radical cation (Scheme III). If nucleophilic attack of a second acetate ion occurs at C-1 (shown in Scheme III) or C-3 (not shown) before the final one-electron reduction, 1,6-$(OAc)_2$BP and 3,6-$(OAc)_2$BP are obtained. The formation of the two diacetoxy derivatives can also occur via one-electron oxidation of 6-OAcBP (Scheme II). Small amounts of 6,12-$(OAc)_2$BP are thought to be formed, but completely converted to BP 6,12-dione.

The high percentage of BP diones, predominantly BP 1,6-dione and 3,6-dione and trace amounts of BP 6,12-dione, from 6-FBP, compared to manganic oxidation of BP, can be rationalized in terms of the high percentage of diacetoxyBPs formed. In fact, the products 1,6-

Scheme IV. Electrophilic Substitution of 6-FBP



$(OAc)_2BP$ and 3,6-$(OAc)_2BP$ by manganic oxidation efficiently yield BP 1,6- and 3,6-dione (Table IV).

The most important consequence of these experiments is the substitution of the fluoride ion at C-6 by another nucleophile after one-electron oxidation of 6-FBP. This indicates that C-6 remains the most reactive under these conditions. A similar result was obtained by electrochemical oxidation of 2- and 4-fluoroanisole in the presence of KOAc; this reaction yielded 2- and 4-acetoxyanisole.[57-59] The mechanism for fluoro displacement can be considered an electron-transfer chain catalysis triggered by a one-electron transfer reagent.[58] More specifically, this mechanism, which involves a radical cation, has been called $S_{ON}2$, a bimolecular nucleophilic substitution via one-electron oxidation of the substrate. Experimental evidence for this $S_{ON}2$ reaction was obtained by Eberson and Jonsson.[59]

To demonstrate that displacement of the fluoro substituent is the result of one-electron oxidation of 6-FBP and not of electrophilic attack, the hydrocarbon was reacted under typical electrophilic conditions. Reaction of 6-FBP with CF_3COOD or pyridinium bromide perbromide (Scheme IV) produced 1,3-dideuteriated 6-FBP or a mixture of 1-Br-6-FBP and 3-Br-6-FBP, respectively, with retention of fluorine at C-6. Thus, displacement of fluorine from C-6 of 6-FBP can occur only via formation of an intermediate radical cation.

6-ClBP and 6-BrBP. Manganic oxidation of 6-ClBP and 6-BrBP yielded mixtures of 1-OAc and 3-OAc derivatives, BP 1,6-dione and 3,6-dione, and a large proportion of starting material (Table I, Scheme III). Formation of the 1-OAc and 3-OAc derivatives of 6-ClBP and 6-BrBP as the most abundant products (Table I) occurs because these are the positions of second highest charge density in $BP^{\bullet+}$ after C-6.[44] These results suggest that the rate of nucleophilic substitution at C-6 in 6-$ClBP^{\bullet+}$ and 6-$BrBP^{\bullet+}$ is lower than in 6-$FBP^{\bullet+}$. Formation of BP 1,6-dione and BP 3,6-dione implies that these quinones can arise via initial attack of the acetate ion at C-6 in 6-$XBP^{\bullet+}$ and/or in 1- and 3-OAc-6$XBP^{\bullet+}$ (Scheme III). The former path is similar to the one for 6-FBP in the formation of diacetoxy derivatives while the latter is postulated upon the significant amount of 1- and 3-OAc-6XBP produced.

Reaction of a mixture of 1- and 3-OAc-6ClBP with $Mn(OAc)_3$ under standard conditions yielded 25% quinones with the remaining as starting material. This result suggests that quinones can be formed via attack of the acetate ion at C-6 in 1- and 3-OAc-6$XBP^{\bullet+}$. The failure to isolate 6-OAcBP (except in traces from 6-ClBP) does not preclude the alternative pathway, i.e., attack of the acetate ion at C-6 in 6-$XBP^{\bullet+}$. In fact, this result is presumably due to the faster rate of formation of diacetoxyBP from 6-OAcBP. Similarly, the failure to isolate diacetoxyBPs can be attributed to the faster rate of decomposition of the diacetoxy derivatives to diones (Scheme II).

In conclusion, evidence indicates that quinones are formed via acetate ion attack at C-6 in 1- and 3-OAc-6$XBP^{\bullet+}$. Although experimental evidence cannot be provided, it is not excluded that a contributory effect to the formation of quinones can derive from acetate ion substitution at C-6 in 6-$XBP^{\bullet+}$.

The high percentage of unreacted starting material from both hydrocarbons is explained by the fact that Cl^- and Br^-, released following nucleophilic attack of acetate ion at C-6 in the pathway leading to quinones, are oxidized by $Mn(OAc)_3$, thereby decreasing the equivalents of $Mn(OAc)_3$ available for oxidation of 6-ClBP and 6-BrBP. In fact, in the presence of NaCl or NaBr, $Mn(OAc)_3$ is reduced efficiently to $Mn(OAc)_2$.

The overall conclusion from manganic oxidation of 6-ClBP and 6-BrBP is that the rate of nucleophilic substitution at C-6 in 6-$ClBP^{\bullet+}$ and 6-$BrBP^{\bullet+}$ is lower than that of $BP^{\bullet+}$ and 6-$FBP^{\bullet+}$.

6-CH_3BP. One-electron oxidation of 6-CH_3BP yielded as the major product the 6-acetoxy derivative linked to the methyl group (Table I, Scheme V). In this case, charge localization at the 6 position produces loss of a proton from the methyl group with formation of a benzylic radical. Further one-electron oxidation affords a benzylic carbenium ion, which yields 6-$OAcCH_2BP$ by nucleophilic attack of an acetate ion. This mechanism has been described for manganic oxidation of *p*-methoxytoluene.[60,61] Some competitive substitution also occurred at positions 1 and 3 with formation of the corresponding monoacetoxy derivatives. In addition, 1-OAc-6-$OAcCH_2BP$ and 3-OAc-6-$OAcCH_2BP$ (8%) were formed as secondary oxidation products. A small amount of 6-$HOCH_2BP$ was also obtained, but this compound may have been formed by hydrolysis of the corresponding acetoxymethyl group on the silica gel column.

An additional observation was made concerning the mixture of the 1- and 3- isomers obtained from manganic oxidation of 6-FBP, 6-ClBP, 6-BrBP, and 6-CH_3BP (see the Experimental Section). The 1-isomer consistently was 65–75% of the mixture. Thus, these two positions have the second and third, highest charge localization in the $BP^{\bullet+}$. Experimental data have shown that the spin density in the high occupied molecular orbitals of BP is 22% greater at C-1 than at C-3.[62] Assuming that the charge density follows the same pattern of the spin density, these data would correlate with the relative acetoxylation at C-1 and C-3 in the 6-substituted $BP^{\bullet+}$.

Conclusions and Biological Significance

Nucleophilic substitution of 6-$FBP^{\bullet+}$, generated by manganic oxidation of 6-FBP, occurs exclusively at position 6, similarly to $BP^{\bullet+}$. For 6-$ClBP^{\bullet+}$ and 6-$BrBP^{\bullet+}$ nucleophilic substitutiuon at C-6 is more difficult, although Cl^- and Br^- are better leaving groups than F^-. Therefore, C-1 and C-3, which are the positions of second highest charge

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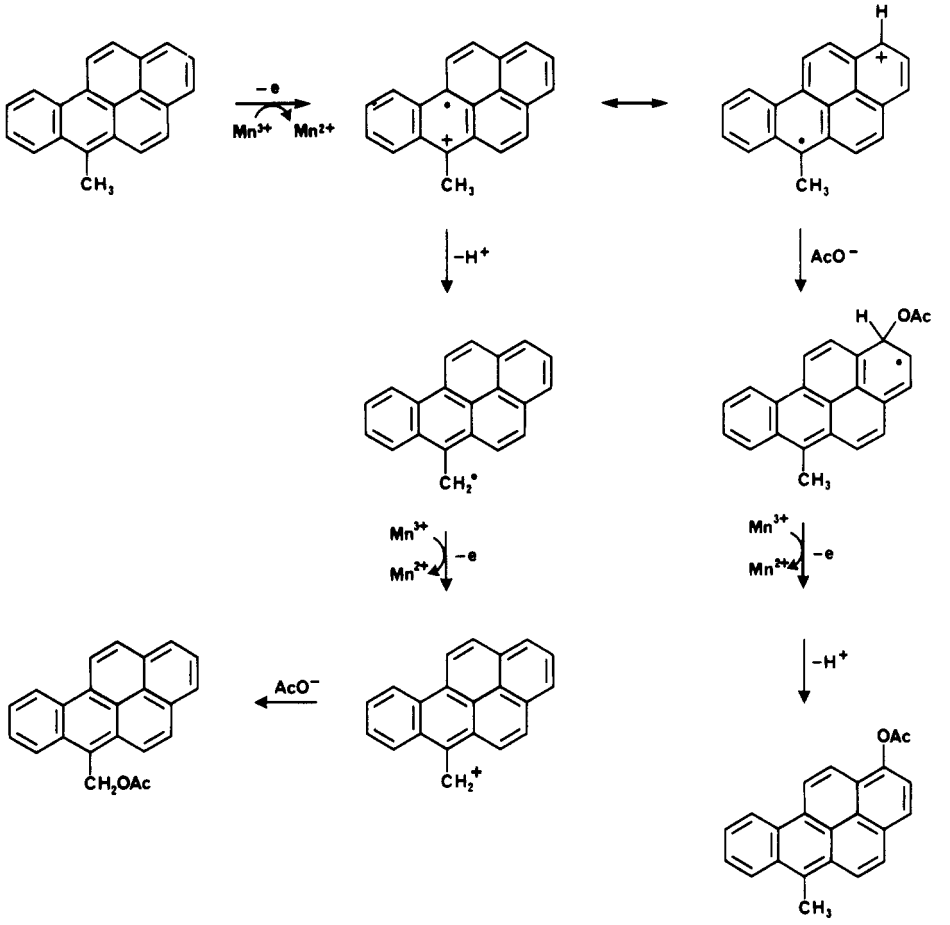
Scheme V. Nucleophilic Substitution in 6-$CH_3BP^{\bullet+}$ Generated by Manganic Oxidation of 6-CH_3BP[a]



[a] Formation of 3-acetoxy and diacetoxy derivatives of 6-CH_3BP not shown.

density in 6-$ClBP^{\bullet+}$ and 6-$BrBP^{\bullet+}$, compete successfully for nucleophilic substitution.

The lower reactivity for 6-$ClBP^{\bullet+}$ and 6-$BrBP^{\bullet+}$ vs 6-$FBP^{\bullet+}$ at C-6 can be in part due to steric factors determined by the bulky chloro and bromo substituents. Alternatively, in terms of inductive effect, halogens are electronegative atoms and tend to destabilize the positive charge at C-6.[63] However, this destabilizing effect is more than compensated for the fluoro substituent by the back-donation from the unshared electron pair of the halogen into the C-6 carbenium ion empty p orbital.[64] Thus, the higher reactivity of 6-$FBP^{\bullet+}$ vs 6-$ClBP^{\bullet+}$ and 6-$BrBP^{\bullet+}$ at C-6 could be accounted for by this latter effect.

For 6-$CH_3BP^{\bullet+}$ charge localization at C-6 activates the methyl group, rendering it the most reactive to nucleophilic attack. However, in this case, acetoxylation at C-1 and C-3 also occurs to some extent.

Displacement of fluorine in the metabolism of 6-FBP by cytochrome P-450,[23,65] horseradish peroxidase, and prostaglandin H synthase[44] to form BP quinones suggests that this process proceeds via radical cation intermediates. This reaction also takes place in the metabolism of 6-ClBP and 6-BrBP, although only minor amounts of BP quinones are obtained.[23] Thus, the metabolic formation of quinones reveals a great similarity between BP and 6-FBP that is consistent with the similar reactivity of these two compounds at C-6 following manganic oxidation. In contrast, 6-ClBP and 6-BrBP, which behave similarly by manganic oxidation, also behave similarly in metabolic formation of BP quinones by cytochrome P-450.

In mouse skin and rat mammary gland, BP, 6-FBP, and 6-CH_3BP are carcinogenic, although the latter two are less potent than BP.[9,45] Conversely, 6-ClBP and 6-BrBP are very weak or virtually inactive.[45] These results reflect the lesser ability of these compounds to undergo nucleophilic substitution at C-6 following one-electron oxidation.

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