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Radical cations of benzo[*a*]pyrene and 6-substituted derivatives: reaction with nucleophiles and DNA

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1. Oxidation of benzo[*a*]pyrene (BP) by I_2 in the presence of $AgClO_4$ in benzene generates the $BP^{\cdot+}ClO_4^- \cdot AgI$ complex. This same method was used to produce radical cations from 6-FBP, 6-ClBP, 6-BrBP and 6-CH_3BP.

2. Reaction of the BP, 6-FBP, 6-ClBP and 6-BrBP radical cation perchlorates with H_2O produced BP 1,6-, 3,6- and 6,12- dione, whereas 6-$CH_3BP^{\cdot+}ClO_4^- \cdot AgI$ yielded 6-CH_2OHBP.

3. When $BP^{\cdot+}ClO_4^- \cdot AgI$ and 6-$FBP^{\cdot+}ClO_4^- \cdot AgI$ were reacted with NaOAc in H_2O/CH_3CN (9:1), 6-OAcBP was formed, in addition to the quinones. In the case of 6-$ClBP^{\cdot+}ClO_4^- \cdot AgI$, a small amount of 1-OAc-6-ClBP and 3-OAc-6-ClBP was formed in addition to the diones, whereas for 6-BrBP and 6-CH_3BP the reaction products were BP diones and 6-CH_2OHBP respectively.

4. These results confirm the localization of charge in the $BP^{\cdot+}$ at C-6, followed by C-1 and C-3.

5. The reaction of BP with $NOBF_4$ in CH_2Cl_2 produced $BP^{\cdot+}BF_4^-$, radical cation free of complexation with inorganic salts.

6. Reaction of $BP^{\cdot+}BF_4^-$ with DNA produced the depurinating adducts BP-6-C8Gua, BP-6-C8dGua and BP-6-N7Gua.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) undergo two main pathways of bioactivation: one-electron oxidation and monooxygenation (Cavalieri and Rogan 1984, 1992). The former yields radical cations, the latter produces oxygenated metabolites.

Many oxygenated metabolites of PAH carcinogens, e.g. diol epoxides, have been synthesized and reacted with DNA. Direct reaction of radical cations with DNA has not been investigated because stable PAH radical cations were not available. To establish radical cations as key intermediates in metabolic activation of PAHs, several chemical approaches have been investigated. Electrochemical oxidation of benzo[*a*]pyrene (BP) in the presence of deoxyguanosine (dG) or deoxyadenosine (dA) forms adducts in which BP is bound at C-6 to the nucleoside (Rogan *et al.* 1988, RamaKrishna *et al.* 1992). In addition, manganic ion oxidation of BP in acetic acid shows that nucleophilic attack of acetate ion occurs regioselectively at C-6, the position of greatest charge density in the BP radical cation (Cremonesi *et al.* 1989).

Metabolic formation of BP quinones catalysed by P450 (Cavalieri et al. 1988b), horseradish peroxidase or prostaglandin H synthase (Cavalieri *et al.* 1988a) occurs

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via the BP radical cation intermediate. Moreover, most of the adducts obtained from the binding of BP to DNA, catalysed by horseradish peroxidase or rat liver microsomal P450, have been identified as the same adducts obtained via electrochemical oxidation of BP in the presence of dG or dA (Cavalieri *et al.* 1990, Devanesan *et al.* 1992, RamaKrishna *et al.* 1992).

In situ generation of PAH radical cations has been useful in identifying these key intermediates as biological metabolites. A valid alternative is to synthesize the radical cation as a solid salt, isolate it, and investigate its reaction with various nucleophiles, including DNA.

Synthesis of radical cation perchlorates by oxidation with I_2 and $AgClO_4$ or radical cation tetrafluoroborates by oxidation with $NOBF_4$ has previously been successful only for perylene (Ristagno and Shine 1971) and thianthrene (Murata and Shine 1969). We have recently reported the synthesis of the radical cation perchlorates and tetrafluoroborates of BP and 6-substituted BPs (Cremonesi *et al.* 1994). We report here the reaction of both perchlorates and tetrafluoroborates of BP and 6-substituted BP radical cations with various nucleophiles or DNA. These studies have been conducted to understand better the role of these intermediates in biological systems.

Materials and methods

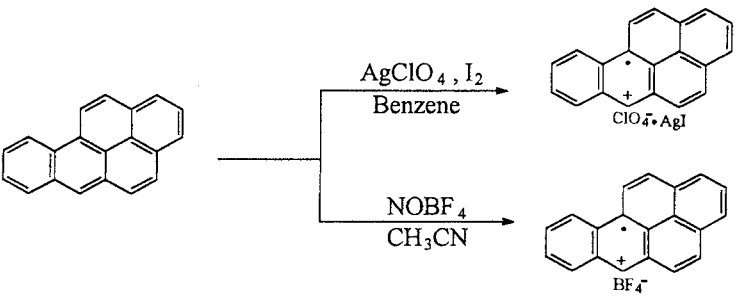
The synthesis of radical cation perchlorates and tetrafluoroborates has been reported in detail elsewhere (Cremonesi *et al.* 1994). Methods used for reaction of radical cations with nucleophiles have also been described previously (Cremonesi *et al.* 1994).

To react radical cations with DNA, approximately 3 mg freshly prepared $BP^{\cdot+}BF_4^-$ in CH_3CN was added to calf thymus DNA (10 mg) in10 ml 0·067 M sodium–potassium phosphate buffer. The mixture was stirred for 30 min and the DNA precipitated by the addition of two volumes of ethanol. The supernatant fraction containing the depurinating adducts was evaporated under vacuum. The residue was dissolved in 1 ml 50:50 methonol:DMSO mixture, filtered and analysed by hplc by using a YMC ODS-AQ 5 μm, 120-Å column (6·0 × 250 mm; YMC, Overland Park, KS, USA) with a flow rate of 1 ml/min. Initial separation was achieved with a methanol/H_2O gradient (30% methanol in H_2O for 20 min, followed by a convex gradient to 100% methanol in 75 min) with detection by a Jasco FP-920 fluorescence monitor. Peaks matching retention times of standard depurinating adducts were collected (solvents were removed and residue was redissolved in the methanol:DMSO mixture) and re-injected in an ethanol/H_2O gradient (30% ethanol in H_2O for 5 min, followed by a linear gradient to 100% ethanol in 75 min). Peaks were collected again and purified by isolation on an isocratic ethanol/H_2O solvent system (58% ethanol (v:v) for BP-6-C8Gua, 61% ethanol for BP-6-N7Gua and BP-6-N7Ade and 67% ethanol for BP-6-N3Ade). After final collection, adduct peaks were dried and analysed by fluorescence line narrowing spectroscopy in the laboratory of Dr G. J. Small (Department of Chemistry and Ames Laboratory, USDOE, Iowa State University).

Results and discussion

Synthesis of radical cations

The isolated yields (amount of black solid recovered), percentage of radical cation (determined by iodometric titration), and yield of radical cations of BP and several 6-substituted BPs are shown in figure 1. Reactions were conducted under argon using anhydrous solvents. The radical cations were isolated as black solids and could be handled in the open for short periods. When stored at − 20°C under argon in solid form, both radical cation perchlorates and tetrafluoroborates were stable for months. A detailed account of methods used for both the assay and identification of these radical cation salts has been presented previously (Cremonesi *et al.* 1994).



Radical cation	% Black solid recovered[a]	% Iodometric assay[b]	% Radical cation formed[c]
$BP^{\cdot+}ClO_4^- \cdot AgI$	90	51	46
$6\text{-}FBP^{+}ClO_4^- \cdot AgI$	63	59	37
$6\text{-}ClBP^{\cdot+}ClO_4^- \cdot AgI$	64	50	32
$6\text{-}BrBP^{\cdot+}ClO_4^- \cdot AgI$	62	52	32
$6\text{-}CH_3BP^{\cdot+}ClO_4^- \cdot AgI$	46	69	31
$BP^{\cdot+}BF_4^-$	90	87	78

[a] Based on respective molecular weight of the radical cation, which includes AgI for the perchlorate salts.
[b] Per cent radical cation contained in the black solid isolated.
[c] Overall yield of radical cation formed from parent PAH.

Figure 1. Isolated yields and iodometric assays of benzo[*a*]pyrene and 6-substituted benzo[*a*]pyrene radical cations.

Reaction with nucleophiles

Nucleophilic reactions have been conducted in both aqueous and anhydrous systems. The former employed water-soluble nucleophiles (inorganic salts, such as sodium acetate) in an aqueous/organic medium (CH_3CN/H_2O 1 : 9) to solubilize both the radical cation salts and the nucleophiles (table 1). The latter used organic nucleophilic salts (tetra-alkylammonium salts) in an anhydrous organic medium (CH_3CN).

Reactions conducted in the presence of H_2O always produced a mixture of 1,6-, 3,6- and 6,12-diones as by-products of the radical cations of BP and 6-substituted BP (table 1). When $6\text{-}CH_3BP^{\cdot+}ClO_4^-$ was reacted in aqueous systems, the major product obtained was $6\text{-}CH_2OHBP$. The data indicate that nucleophilic attack of H_2O occurs at C-6 in the radical cation of BP and 6-halogeno derivatives and at the 6-methyl group in $6\text{-}CH_3BP^{\cdot+}ClO_4^-$. These results are in agreement with those obtained by manganic ion oxidation of the respective PAHs (Cremonesi *et al.* 1989) or by anodic oxidation of BP in H_2O (Jeftic and Adams 1970). The relatively weak nucleophile OAc^- displayed high selectivity with $BP^{\cdot+}ClO_4^-$ at C-6 (table 1), the position of greatest charge density in $BP^{\cdot+}$ (Sullivan *et al.* 1985, Cremonesi *et al.* 1989)

In principle, the anhydrous medium would be expected to yield effective nucleophilic reactions. However, the limitation in this case is the tendency of radical cations to form dimeric products. In the case of both OAc^- and F^- nucleophiles, the yield of substituted products was higher than those reactions performed in an aqueous environment (table 1). The formation of quinones was generally $< 1\%$, but formation of dimers was detected by both tlc and hplc.

Table 1. Nucleophilic substitution on radical cations of benzo[*a*]pyrene (BP) and 6-substituted BP.

	Nucleophiles[a]							
Radical cation	H_2O	% Yield	NaOAc	% Yield	$(CH_3)_4NOAc$	% Yield	$(CH_3)_4NF{\cdot}2HF$	% Yield
$BP^{\cdot+}ClO_4^-$	diones	29	6-OAcBP	11	6-OAcBP	17	6-FBP	25
			diones	29	diacetates	3		
					diones	1		
$6\text{-}FBP^{\cdot+}ClO_4^-$	diones	31	6-OAcBP	1				
			diones	35				
$6\text{-}ClBP^{\cdot+}ClO_4^-$	diones	14	(1,3)-OAc-ClBP	3				
			diones	12				
$6\text{-}BrBP^{\cdot+}ClO_4^-$	diones	10	diones	12				
$6\text{-}CH_3BP^{\cdot+}ClO_4^-$	$6\text{-}CH_2OHBP$	5	$6\text{-}CH_2OHBP$	5	$6\text{-}CH_2OAcBP$	6		
					$6\text{-}CH_2OHBP$	17		
$BP^{\cdot+}BF_4^-$					6-OAcBP	18	6-FBP	20
					diacetates	2		
					diones	3		

[a] Nucleophile reactions with H_2O and NaOAc were conducted in a CH_2CN/H_2O (1:9) solvent system, while nucleophile reactions with $(CH_3)_4NOAc$ and $(CH_3)_4NF{\cdot}2HF$ were conducted in anhydrous CH_3CN.

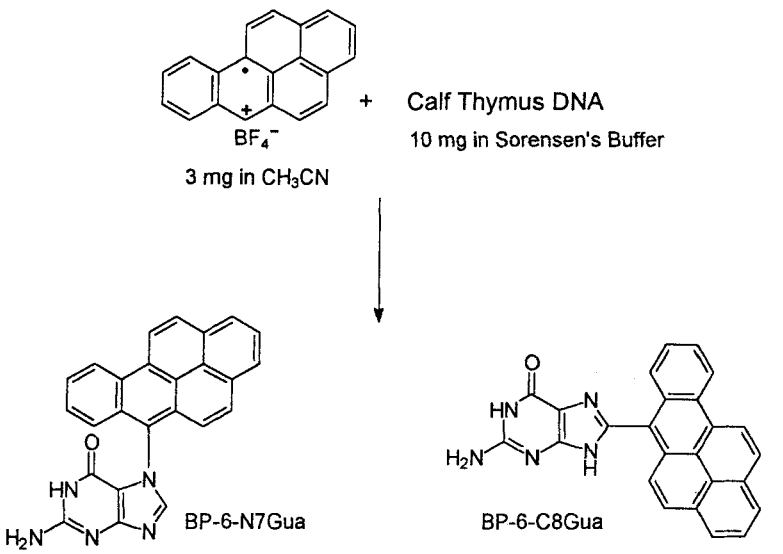


Figure 2. Depurinating adducts formed by reaction of $BP^{\cdot+}BF_4^-$ with DNA.

Reaction with DNA

When the binding of BP to DNA is catalysed by horseradish peroxidase (HRP) (Cavalieri *et al.* 1988b) or rat liver microsomes (Cavalieri *et al.* 1988a), the major depurinating adducts formed are BP-6-N7Ade, BP-6-C8Gua, and BP-6-N7Gua. The reaction of $BP^{\cdot+}BF_4^-$ with DNA results in the formation of two depurinating adducts: BP-6-N7Gua and BP-6-C8Gua (figure 2). Analysis of the stable adducts by the ^{32}P-postlabelling method shows that the radical cation generates an adduct profile identical to that seen after HRP activation of BP (Todorovic *et al.* 1993), an expected result because HRP activates BP via one-electron oxidation (Rogan *et al.* 1979, 1988).

Conclusions

Radical cations of BP and 6-substituted derivatives were synthesized by reaction of the PAH in benzene with I_2 and $AgClO_4$, or by reaction in CH_2Cl_2/CH_3CN with $NOBF_4$. The radical cation perchlorates were isolated as complexes with AgI, whereas the tetrafluoroborates were free of AgI and, therefore, displayed greater solubility in organic solvents; both were stable for prolonged periods when stored at subzero temperatures under argon. When these radical cations are reacted with nucleophiles of various strength, the pattern of nucleophilic substitution reflects the distribution of positive charge localization in the radical cation. Higher selectivity for the position of highest charge localization, C-6, is displayed by H_2O, ACO^- and F^-. Reaction with DNA produces two depurinating adducts, BP-6-N7Gua and BP-6-C8Gua.

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