Oxidized metabolites from benzo[*a*]pyrene, benzo[*e*]pyrene, and aza-benzo[*a*]pyrenes. A computational study of their carbocations formed by epoxide ring opening reactions

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A DFT study aimed at understanding structure–reactivity relationships and fluorine substitution effects on carbocation stability in benzo[*a*]pyrene (BaP), benzo[*e*]pyrene (BeP), and aza-benzo[*a*]pyrene (aza-BaP) derivatives are reported. The relative energies of the resulting carbocations are examined and compared, taking into account the available biological activity data on these compounds. *O*-Protonation of the epoxides and diol epoxides leads to carbocation formation by barrierless processes. Charge delocalization modes in the resulting carbocations were deduced *via* NPA-derived changes in charges, and fluorine substitution effects were analyzed on the basis of charge density at different carbocation positions. Thus, fluorine substitution at sites bearing negative charge generated inductive destabilization of the carbocation, whereas a fluorine atom at a ring position which presented significant positive charge density produced a less pronounced destabilization due to fluorine p–π back-bonding. Protonation reactions were also studied for the azaBaPs. In selected cases, the covalent adducts generated *via* bond formation with the exocyclic nitrogen of cytosine were computed and relative energies and geometries of the resulting adducts were examined.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants.[1] They exert their mutagenic and/or carcinogenic activity through metabolic activation pathways, principally *via* formation of bay-region diol epoxides (DEs).[2] Benzylic carbocations generated from these electrophilic DEs by opening of the *O*-protonated epoxide ring are capable of forming covalent adducts with the nucleic acids.[2] Adduct formation by reaction with the nucleophilic sites in DNA and RNA is a key step in the mechanism by which PAHs can initiate the alteration of genetic material.[2]

Benzo[*a*]pyrene (BaP), one of the most highly investigated environmental contaminants, exhibits potent carcinogenicity and mutagenicity effects.[2,3] Its ultimate active metabolite is the bay-region 7,8-dihydrodiol-9,10-epoxide-2 diastereomer (DE-2), in which the 7-hydroxyl group is *trans* to the 9,10-epoxide oxygen (the DE-1 diastereomer has the *cis* disposition).[4] By contrast, both diastereomers of benzo[*e*]pyrene (BeP) bay-region 9,10-diol-11,12-epoxide have little tumorigenic activity.[5] Structures and numbering are displayed in Fig. 1.

Mechanistic studies on the hydrolysis of BaP-DE demonstrated pH dependency and catalysis by DNA and by polynucleotides, showing that protonation must occur either before or during the rate determining step.[6] Based on these studies, it was proposed that a DNA-intercalated DE reacts to form a benzylic carbocation in the rate determining step. The monohydrogenphosphate group on the nucleotide can act as general acid, and stacking interactions between the PAH-DE and the base contribute to catalysis. Moreover, it is likely that electrophilic attack of DNA nucleotides by PAH epoxides is S_N1-like and proceeds through proton-stabilized transition states in which the hydrocarbon exhibits significant carbocationic character.[7]

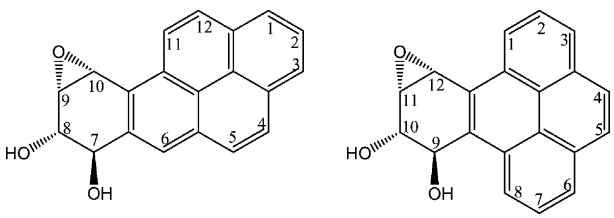

Fig. 1 Structures and numbering of active metabolites of BaP and BeP.

The carcinogenic activity of PAHs is often strongly affected by fluorine substitution at strategic molecular sites.[2,8] In the case of BaP, monosubstitution by fluorine at positions 7, 8, 9 and 10 resulted in mutagenic compounds, the 10-F derivative presenting some decrease in mutagenicity, while a several-fold increase in mutagenicity occurred when fluorine was present at position 8.[9] In contrast, the 6-F-BaP-DE had no significant tumorigenic activity.[10]

Despite the numerous reports on BaP carcinogenesis, only a few studies have been carried out on the toxicity of aza-benzo[*a*]pyrenes (azaBaPs). In case of 10-azaBaP, an analogue with a nitrogen atom in the bay region, no bay-region DE could be formed. This compound has been reported to be carcinogenic,[11] although less mutagenic *in vivo* than BaP.[12] Its major metabolite is the 4,5-oxide, for which isolation of cytosine adducts have been reported.[13] Moderate mutagenicity has been reported for 6-azaBaP.[14]

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Quantum-mechanical calculations have shown very good agreement with the experimental reactivities of several PAH and aza-PAH metabolites, when applied to the study of carcinogenic pathways of these compounds.[15] Additionally, modeling studies on biological electrophiles from PAHs by density functional theory (DFT) methods have yielded appropriate descriptions of the NMR features and charge delocalization modes in their resulting carbocations.[16]

In this work we have performed a model DFT study on the structural and electronic properties of the electrophilic reactive intermediates of BaP, BeP and azaBaP derivatives, for which changes in energy for epoxide ring opening reactions were calculated. Conformational features and their relationship to reactivity were examined. Fluorine substitution at selected molecular sites was investigated. Charge delocalization modes (positive charge density distribution) in the resulting carbocations were evaluated by means of the NPA-derived changes in charges (carbocation minus neutral). Protonation reactions were also analyzed for the aza-BaP compounds.

In our previous studies, consideration of the solvent effect by means of PCM calculations did not afford any significant variation compared to gas-phase reactivity trends.[15] Whereas reaction energies were certainly influenced by solvation, the reactivity orders for the series of compounds remained unchanged. Since the goal of the present study was to determine relative reactivities rather than to compute absolute reaction rates, only gas-phase calculations were carried out as a way to reduce the computational costs.

To model the crucial step of covalent adduct formation, adducts resulting from quenching of 10-azaBaP-4,5-epoxide with cytosine *via* the exocyclic amino group were computed, and their geometrical features and relative energies were compared.

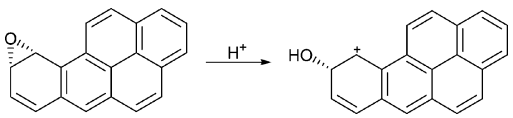
Computational methods

Density functional theory (DFT) calculations were performed with the Gaussian 03 package,[17] employing the B3LYP functional[18] and the 6-31G* split-valence shell basis set. The diffuse- and polarization-function-augmented 6-31+G** basis set was also used in representative cases for comparison. Geometries were fully optimized and minima were characterized by calculation of the harmonic vibrational frequencies. Natural bond orbital population analysis (NPA) was evaluated by means of the NBO program.[19]

Results and discussion

Benzo[*a*]pyrene derivatives

Changes in energy for the epoxide ring opening reactions of *O*-protonated BaP-9,10-dihydroepoxide (reaction (1)) and BaP-7,8-dihydrodiol-9,10-dihydroepoxide-2 were calculated and the results are summarized in Table 1. The protonated epoxides, that is, the oxonium ions, could not be located as minima on the respective potential energy surfaces because the epoxide ring opened by a barrierless process upon *O*-protonation. The same behavior was observed for every protonated epoxide in this study. Calculations at the B3LYP/6-31+G** level gave rather similar results to those afforded *via* B3LYP/6-31G*. Therefore, the less computationally expensive 6-31G* basis set was employed for the entire study.



(1)

For the *trans*-diol structure (BaP-DE), the conformation with both hydroxyl groups in a pseudoequatorial arrangement was more stable than the pseudoaxial form by 4 kcal mol^{-1}. This was true for both the DE and its derived open triol carbenium ion, in accordance with the previously reported observations.[20] Tables and figures throughout this work will always refer to the conformationally most favored (lowest energy) DE-2 diastereomer of each compound.

Charge delocalization maps are shown in Fig. 2. According to the NPA-derived charge distributions, positive charge in the resulting carbocations was delocalized throughout the π-system. The epoxide and DE afforded similar delocalization patterns for the resulting carbocations.

The effect induced by fluorine substitution on ΔE_r for the epoxide opening reaction, and hence on carbocation stabilities, was analyzed for various positions not involving the DE framework. According to the charge delocalization maps for both bay-region epoxides, positions 4, 3 and 1 would be expected to be more influenced by substitution, as they bear the higher positive

Table 1 Calculations for BaP derivatives

Compound	ΔE_r/kcal mol^{-1}	Δq [a]	ΔqC-10 [b]	ΔqF [c]	C–F Bond length/Å [d]
BaP-9,10-epoxide	−243.9 (−243.5)[e]	—	−0.087	—	—
BaP-7,8-diol-9,10-epoxide	−241.0 (−240.1)[e]	—	−0.117	—	—
1-F-BaP-DE	−241.2	0.088	−0.120	0.040	1.330
2-F-BaP-DE	−237.4	0.000	−0.109	0.030	1.335
3-F-BaP-DE	−240.2	0.092	−0.120	0.041	1.330
4-F-BaP-DE	−240.2	0.101	−0.119	0.043	1.328
5-F-BaP-DE	−237.4	−0.012	−0.110	0.025	1.338
6-F-BaP-DE	−239.9	−0.013	−0.095	0.024	1.335
11-F-BaP-DE	−239.2	0.000	−0.103	0.023	1.346[f]
12-F-BaP-DE	−239.6	0.078	−0.116	0.038	1.331

[a] Change in charge density for the indicated carbon atom in the nonfluorinated compound (qC$_{carbocation}$ − qC$_{epoxide}$). [b] Change in charge density at the carbocation center, between the open carbocation and the neutral closed epoxide (qC-10$_{carbocation}$ − qC-10$_{epoxide}$). [c] Change in charge density between the open carbocation and the neutral closed epoxide (qF$_{carbocation}$ − qF$_{epoxide}$). [d] Open carbocation. [e] B3LYP/6-31+G** results. [f] The fluorine atom forms an attractive interaction with H-10.

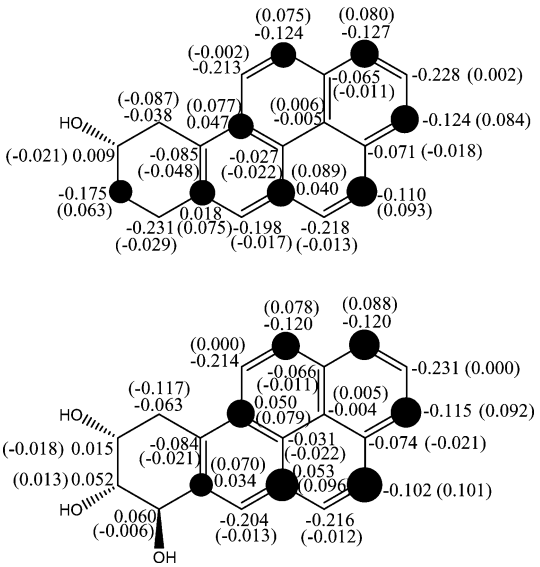


Fig. 2 Computed NPA heavy atom charge densities (Δcharges relative to the neutral compound in parentheses) for the carbocations generated from BaP-9,10-epoxide and bay-region DE. (The dark circles are roughly proportional to the magnitude of C Δcharges; the threshold was set to 0.030.)

charge densities, followed by position 12. The calculated results followed this trend (Table 1). Fluorination was found to reduce the exothermicity of the epoxide opening reaction, and consequently the fluorinated compounds are predicted to be less active than the parent BaP. The decrease in carbocation stability was less pronounced when the fluorine atom was at highly positively charged sites, due to p–π back-bonding and development of fluoronium ion character, as reflected by development of positive charge at F and shortening of the C–F bond (C–F was *ca.* 1.35 Å in the neutral epoxides). On the other hand, F-substitution at a position with negative charge density produced a more pronounced decrease in carbocation stability (with the exception of the 6-F derivative).

For the 11-F derivative, presence of an attractive interaction between fluorine and the hydrogen atom attached to C-10 (the H · · · F distance is *ca.* 2.04 Å) favored epoxide ring opening, despite the absence of positive charge density at that position.

For the 6-F compound, the unusual pseudoaxial conformation was preferred for both the DE and the triol carbenium ion. These observations are in accord with the solution configurations determined by NMR measurements.[21] The 9,10-epoxy-7,8,9,10-tetrahydro derivatives of BaP and 6-F-BaP, which lack the 7,8-diol substituents, have approximately equal mutagenic activity,[21a] and a purely inductive effect of the 6-F substituent was excluded.[21b] Thus, it was suggested that the altered diol conformation of 6-F-BaP-DE, resulting from the adverse interaction of the fluorine with the hydroxyl group at the adjacent C-7 position, could result in inefficient alkylation, or more efficient repair, of the cellular target, pointing to the influence of conformation of hydroxyl groups in bay-region DEs on the expression of mutagenic and tumorigenic activity.[10] Our computational results showed that epoxide opening from the pseudoaxial conformation is more favored by *ca.* 2 kcal mol^{-1} for BaP-DE, and by *ca.* 4 kcal mol^{-1} for 6-F-BaP-DE. In this manner, the lower bioactivity of the pseudoaxial conformations could stem from intercalation issues with the DNA that are not considered in the present calculations. On the other hand, epoxide ring opening of 6-F-BaP epoxide was less favored than 12-F-BaP epoxide, in contrast to the DE case. Hence, in the absence of an interaction between 6-F and 7-OH, the reactivity followed the order inferred from the charge delocalization mode.

BaP-7,8-diol-9,10-epoxide has been reported to be the most mutagenic derivative and the one which forms the major DNA adducts.[9] Therefore, positions 7, 8, 9 and 10 of the BaP structure are subject to metabolic activation to the corresponding DE. As the first three of these sites do not participate in resonance stabilization of the bay-region carbocation, the corresponding monosubstituted fluoro compounds would be expected to have the same activity as the parent BaP. However, as several-fold increase in mutagenicity occurred for 8-F-BaP, the involvement of other metabolites in fluorinated BaP mutagenesis has been suggested.[9] It should be noted that, although 8-F-BaP was found to be more mutagenic than BaP, this fluorinated derivative was much less active than its parent hydrocarbon for the induction of sarcomas.[9] On the other hand, the 10-F derivative presented some decrease in mutagenicity.[9]

In order to check the effect of prior fluorination on the epoxidation and subsequent carbocation formation processes, the changes in energy for reactions in Scheme 1 were computed.

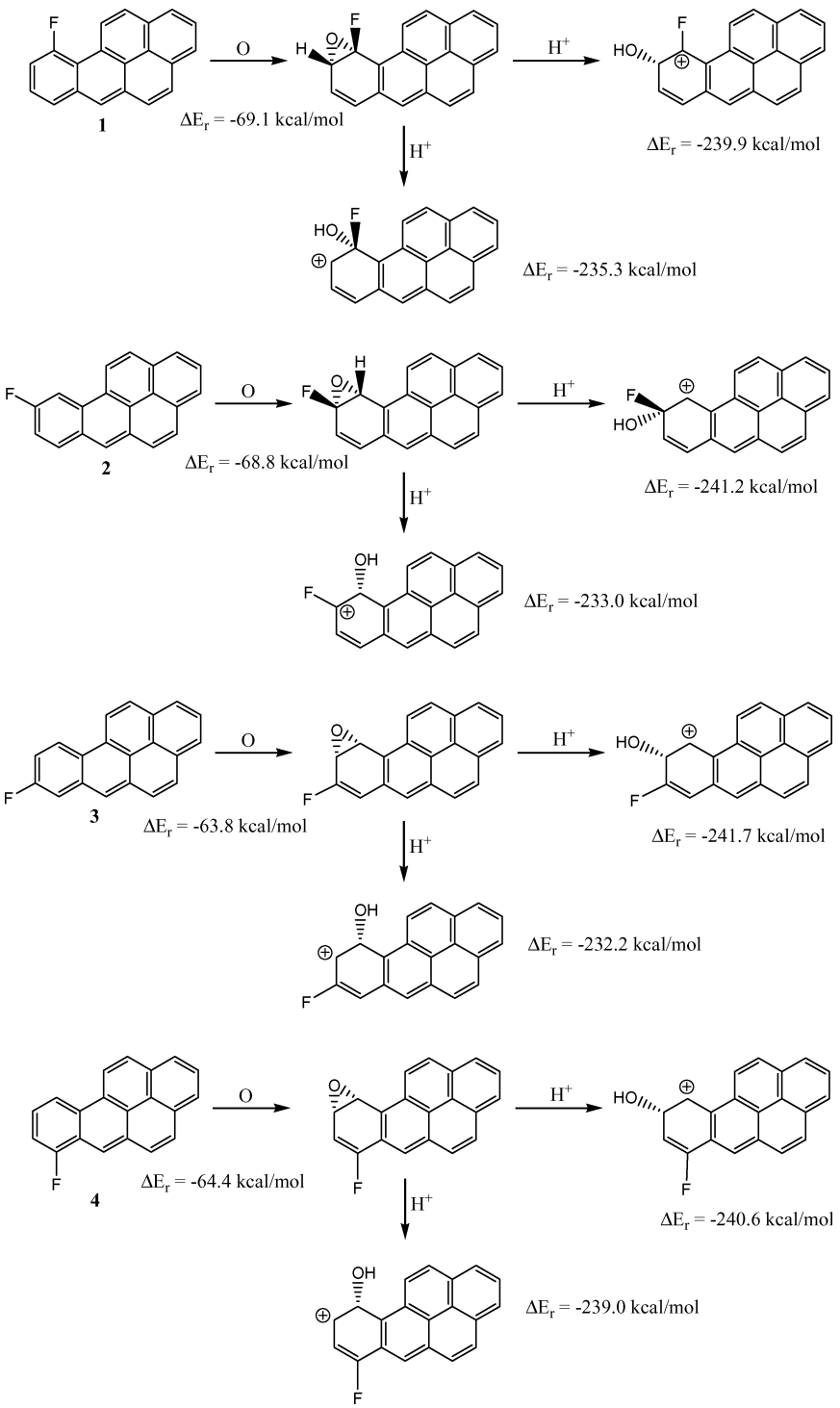
Fluorination did not hinder the epoxidation step, which was exothermic in all cases. The higher exothermicity of the epoxide ring opening for the 8-F derivative (**3**) was consistent with the presence of positive charge density at the 8-position. Stabilization of the generated carbocation by electron density donation from fluorine is thus in accordance with the increased activity of this compound. Moreover, the negative charge development at the bay-region carbocationic center corresponded with diminished exothermicity for the 10-F compound (**1**), which agrees with its lower biological activity.

Benzo[*e*]pyrene derivatives

Reaction of BeP-9,10-diol-11,12-epoxide with DNA resulted in covalent adduct formation, and the extent of covalent binding was found to be lower than the binding of BaP-7,8-diol-9,10-epoxide to DNA under the same experimental conditions.[22] Bay-region BeP-9,10-epoxide presented a high mutagenicity, almost similar to that of BaP-9,10-epoxide.[23] However, the major metabolite produced by BeP was BeP-4,5-dihydrodiol, which derives from the hydrolysis of the weak mutagen BeP-4,5-epoxide.[24] In this way, the low carcinogenicity of BeP was suggested to result from the lack of metabolic formation of their potential ultimate carcinogenic bay-region derivatives.[23,24]

Changes in energy for the epoxide ring opening reactions of *O*-protonated BeP-11,12-dihydroepoxide and BeP-9,10-dihydrodiol-11,12-dihydroepoxide-2 were calculated, and the results are displayed in Table 2. The opening reactions were less exothermic than those for the respective BaP bay-region derivatives, in accord with the lower bioactivity of BeP. The reaction for the K-region BeP-4,5-epoxide was found to be less favored, which also agreed with the mutagenicity assays mentioned above.

In both the bay-region DE of BeP and its open triol carbenium ion, the hydroxyl groups preferred the pseudoequatorial



Scheme 1 Epoxidation and subsequent carbocation formation in fluorinated BaPs.

conformation, and the opening reaction was computed to be more exothermic for this conformation. Based on previous solution studies, it was deduced that both the 1 and 2 diastereomers of BeP-9,10-diol-11,12-epoxide are locked in pseudoaxial conformation due to steric crowding in the bay region.[25] Nevertheless, our computed structures showed that the pseudoequatorial disposition is stabilized by an attractive interaction between the oxygen atom of the hydroxyl at C-9 and the hydrogen attached to C-8, while this interaction is not allowed in the pseudoaxial conformation.

The effect of fluorine substitution on ΔE_r for the epoxide opening reaction was examined for the carbon atoms not involved in the DE structure. Considering the charge delocalization map for the parent carbocation (Fig. 3), fluorine substitution should be more favorable at positions 6 and 8, which bear higher positive

Table 2 Calculations for BeP derivatives

Compound	ΔE_r/kcal mol^{-1}	Δq [a]	C–F bond length/Å [b]
BeP-4,5-epoxide	−230.1	—	—
BeP-11,12-epoxide	−237.9	—	—
BeP-9,10-diol-11,12-epoxide	−238.3	—	—
1-F-BeP-DE	−237.1	0.025	1.348[c]
2-F-BeP-DE	−233.7	0.031	1.335
3-F-BeP-DE	−234.7	0.026	1.338
4-F-BeP-DE	−234.6	0.027	1.339
5-F-BeP-DE	−233.2	0.013	1.340
6-F-BeP-DE	−236.4	0.125	1.324
7-F-BeP-DE	−231.7	−0.016	1.336
8-F-BeP-DE	−233.6	0.097	1.325

[a] Change in charge density for the indicated carbon atom in the nonfluorinated compound ($qC_{carbocation} - qC_{epoxide}$). [b] Open carbocation. [c] The fluorine atom is involved in an attractive interaction with H-12.

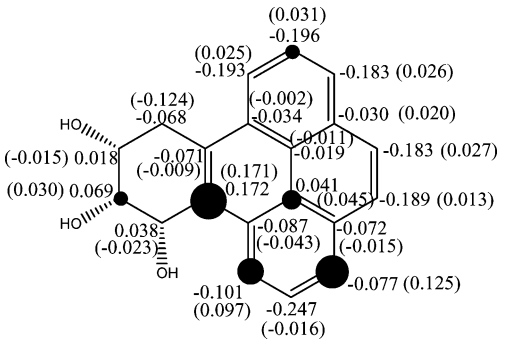


Fig. 3 Computed NPA heavy atom charge densities (Δcharges relative to the neutral compound in parentheses) for the carbocation generated from BeP bay-region DE. (The dark circles are roughly proportional to the magnitude of C Δcharges; the threshold was set to 0.030.)

charge densities, while position 7 should be less favored. The exothermicity of the reactions followed this trend, although the process was not as favorable as expected for the derivative with fluorine at C-8. This finding was explained by the repulsive interaction of the fluorine atom with the oxygen of the hydroxyl group at the adjacent C-9 position. Consequently, for this compound, the diol pseudoaxial conformation was more stable than the pseudoequatorial one for both the DE and its open triol carbenium ion, unlike the other fluorinated BeP derivatives. Nonetheless, the opening reaction for the pseudoaxial conformation was less favored than that for the pseudoequatorial conformer by *ca.* 2 kcal mol^{-1}. In contrast, the epoxide ring opening of 8-F-BeP epoxide was more exothermic than that for 2-F-BeP epoxide. Hence, without the 8-F–9-OH repulsive interaction, the reactivity agreed with the charge delocalization map.

For the 1-F derivative, the epoxide ring opening is assisted by the presence of an attractive interaction between fluorine and the hydrogen atom attached to C-12 (the H···F distance was *ca.* 2.00 Å). In order to achieve a stronger H···F contact without weakening the 9-HO–8-H interaction, 1-F-BeP-DE presented a slight deviation from planarity in comparison with the other fluorinated BeP-DEs. The 1-F-BeP triol-carbenium ion also presented a different conformation than other related fluorinated triol-carbenium ions. Nevertheless, in all cases the pseudoequatorial conformation for the hydroxyl groups was preferred.

Aza-benzo[*a*]pyrene derivatives

Changes in energy for epoxide ring opening reactions for *O*-protonated aza-BaP epoxides and the DE derivatives were computed (Table 3). The studied compounds were 10-azaBaP-4,5-epoxide (K-region epoxide), 4-azaBaP-9,10-epoxide (bay-region epoxide) and 4-azaBaP-7,8-diol-9,10-epoxide (bay-region DE), and 6-azaBaP bay-region epoxide and DE. The effect of the position of the nitrogen atom on the epoxidation step was also evaluated. These reactions were found to be exothermic in all cases (Scheme 2).

The results in Table 3 demonstrate that the most stable carbocations were those derived from 6-azaBaP. Inspection of the charge delocalization patterns (Fig. 4) reveal that this carbocation is highly delocalized and no appreciable positive charge density develops at nitrogen, while the heteroatom becomes less negatively charged in the 4-azaBaP cation. The carbocation derived from the 10-azaBaP K-epoxide was computed to be the least stable. It can be noticed that carbocation stability increases with increased delocalization of the net positive charge through the aromatic system, as indicated by the development of negative charge density at the carbocationic center. Nevertheless, according to these calculations the aza-compounds would be less reactive than BaP, and this is in agreement with the reported mutagenicity assessments.[12,14]

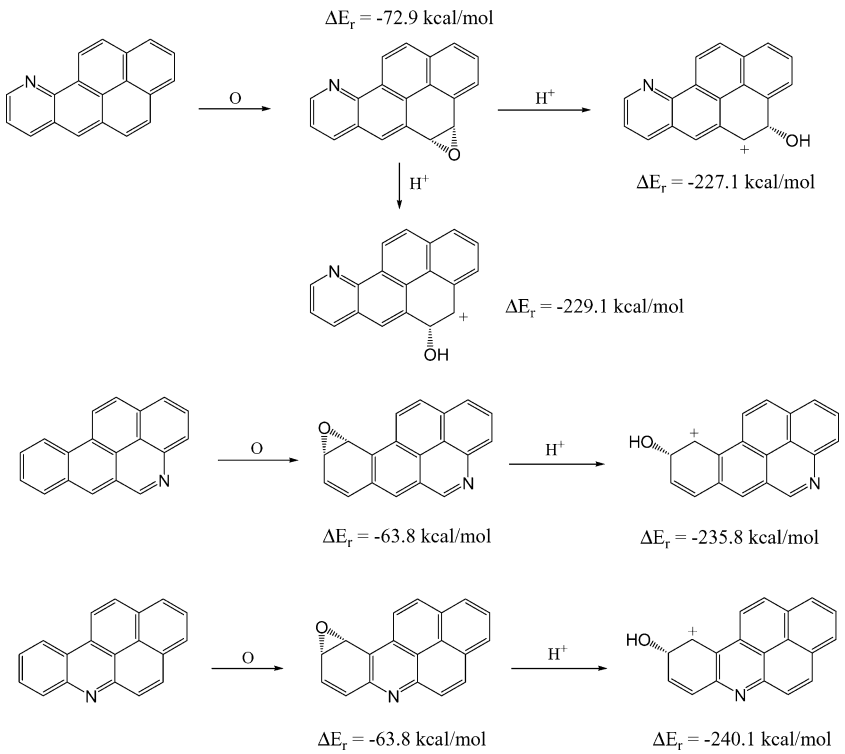
Considering the possibility/feasibility of biological alkylation at the heteroatom in aza-PAHs, the effect of *N*-alkylation was considered in the case of 4- and 10-azaBaP-epoxides. In this way, generation of the corresponding azonium–carbenium dications by ring opening of the protonated epoxide in the *N*-methylated onium ions was analyzed (Scheme 3). Epoxide ring opening was less exothermic for the *N*-methylated derivatives, although it was still barrierless, similar to the previous compounds. Interestingly, whereas the regioselectivity of epoxide ring opening was unchanged in the case of the 10-aza-compound, formation of the non-bay-region dication was the favored process for the 4-aza-derivative.

The fluorine substitution effect on ΔE_r was examined in the case of 4-azaBaP-9,10-epoxide. The epoxide ring opening reaction was analyzed for various regioisomeric fluorinated PAH-epoxides except for sites involved in the bay-region DE structure (C-7 through C-10). The results are gathered in Table 3. Taking into account the charge delocalization map for the carbocation (Fig. 4),

Table 3 Calculations for AzaBaP derivatives

Compound	ΔE_r/kcal mol^{-1}	ΔqN [a]	Δq [b]	C–F Bond length/Å [c]
4-AzaBaP-9,10-epoxide	−235.8	0.073	—	—
4-AzaBaP-7,8-diol-9,10-epoxide	−232.3	0.079	—	—
10-AzaBaP-4,5-epoxide	−229.1	0.014	—	—
6-AzaBaP-9,10-epoxide	−240.1	0.006	—	—
6-AzaBaP-7,8-diol-9,10-epoxide	−236.1	−0.050	—	—
4-AzaBaP-9,10-epoxide-1-F	−235.0	—	0.082	1.330
4-AzaBaP-9,10-epoxide-2-F	−232.4	—	0.000	1.334
4-AzaBaP-9,10-epoxide-3-F	−235.4	—	0.089	1.319
4-AzaBaP-9,10-epoxide-5-F	−232.2	—	−0.016	1.334
4-AzaBaP-9,10-epoxide-6-F	−232.6	—	−0.023	1.341
4-AzaBaP-9,10-epoxide-11-F	−234.6	—	0.000	1.345[d]
4-AzaBaP-9,10-epoxide-12-F	−234.7	—	0.082	1.330

[a] Change in charge density between the open carbocation and the neutral closed epoxide ($q\mathrm{N}_{\mathrm{carbocation}} - q\mathrm{N}_{\mathrm{epoxide}}$). [b] Change in charge density for the indicated carbon atom in the nonfluorinated compound ($q\mathrm{C}_{\mathrm{carbocation}} - q\mathrm{C}_{\mathrm{epoxide}}$). [c] Open carbocation. [d] The fluorine atom is involved in an attractive interaction with H-10.



Scheme 2 Epoxidation and subsequent carbocation formation in azaBaP derivatives.

fluorine substitution should be more favorable at positions 3, 1 and 12, where higher positive charge densities are located. This was the order followed by the exothermicity of the reactions. For the 11-F derivative, an attractive interaction between fluorine and the H-10 (the H · · · F distance was *ca.* 2.07 Å) favored the epoxide ring opening, although no positive charge density developed at that position.

In case of the K-region 10-azaBaP-4,5-epoxide, the regioselectivity of epoxide ring opening was dependent on the position of the fluorine substituent, and the reactions were less exothermic than those of 4-azaBaP-9,10-epoxide (by roughly 5 kcal mol^{-1}).

Furthermore, protonation reactions were computed for every position in 4-azaBaP and 10-azaBaP (Table 4). The heteroatom was the most favored protonation site, in line with the higher basicity of nitrogen. The charge delocalization mode for the most stable *C*-protonated cation derived from 10-azaBaP (protonated at C-6, Fig. 5) was compared with the corresponding one derived from epoxide ring opening (Fig. 4). However, no substantial similarities were found between their charge distributions. This observation suggests that for the studied systems, ring protonation (serving as a model for attack by electrophilic oxygen) and epoxide ring opening (resulting in benzylic carbocations) are not related processes, and cannot be explained by the same factors. Consequently, carbocations derived from protonation may not serve as rational models for bay-region epoxide ring opening carbocations. Instead, *C*-protonation is directed by the

Table 4 Protonation reactions for AzaBaP derivatives

4-AzaBaP		10-AzaBaP	
Protonated position	ΔE_r/kcal mol^{-1}	Protonated position	ΔE_r/kcal mol^{-1}
C-1	−224.5	C-1	−226.6
C-2	−208.6	C-2	−209.4
C-3	−224.4	C-3	−225.8
N-4	−246.6	C-4	−213.1
C-5	−202.9	C-5	−214.5
C-6	−224.5	C-6	−230.9
C-7	−215.2	C-7	−211.1
C-8	−208.3	C-8	−208.5
C-9	−214.1	C-9	−213.0
C-10	−211.0	N-10	−244.2
C-11	−211.3	C-11	−213.5
C-12	−216.5	C-12	−219.1

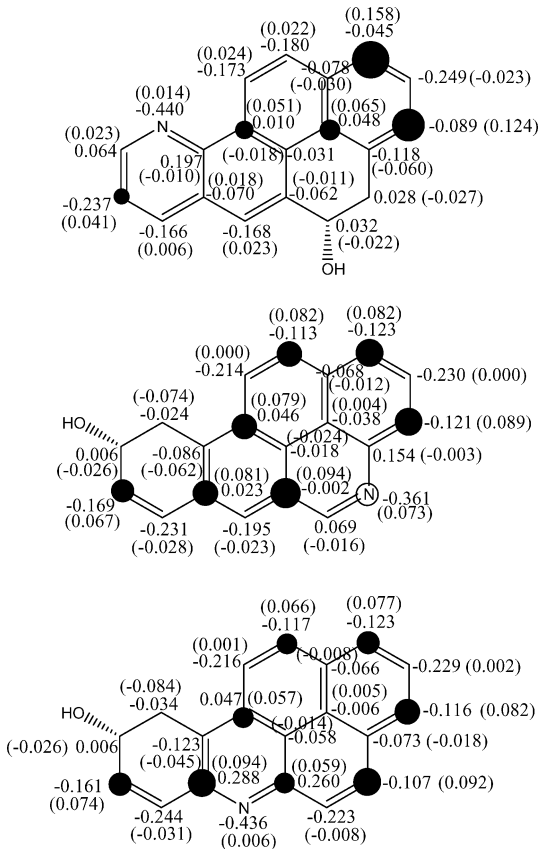
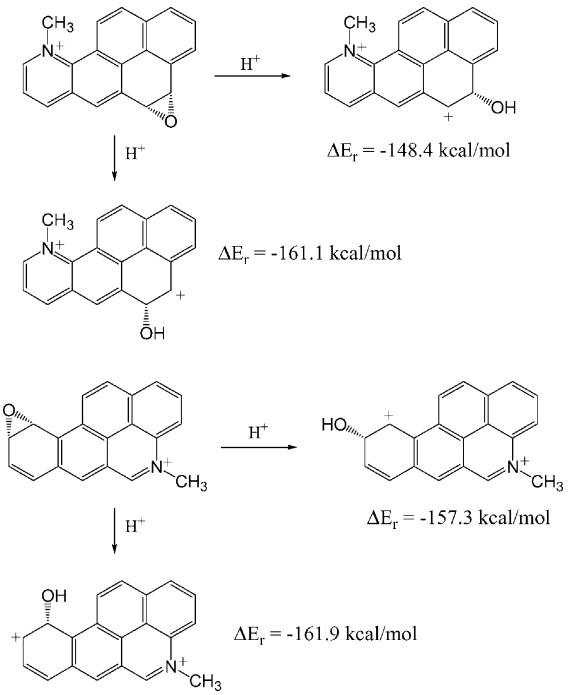


Fig. 4 Computed NPA heavy atom charge densities (Δcharges relative to the neutral compound in parentheses) for the carbocations generated from azaBaP epoxides. (The dark circles are roughly proportional to the magnitude of C Δcharges, and white circles to N Δcharges; the threshold was set to 0.030.)

HOMO configuration in the neutral PAHs, which exhibits higher coefficients at positions 6, 1, 3, and 12 (the same order as calculated changes in energy for the protonation reactions). In keeping with the hard and soft acid and base (HSAB) principle, protonation at a "hard" nitrogen atom is a charge-controlled process, whilst protonation of a "softer" aromatic carbon is orbital-controlled. The HOMOs for both neutral azaBaPs are displayed in Fig. 5.

Isolation of the DNA adducts resulting from the attack of the exocyclic amino group of cytosine at C-4 of 10-azaBaP-4,



Scheme 3 Generation of dications by *N*-methylation and epoxide ring opening in aza-BaP epoxides.

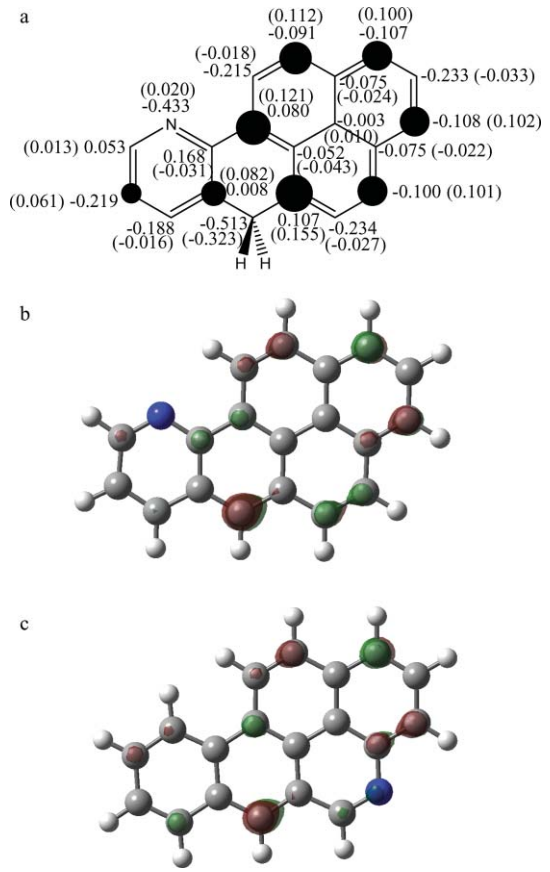


Fig. 5 (a) Computed charge densities (Δcharges relative to the neutral compound in parentheses) for the C-6-protonated 10-azaBaP cation. (b) HOMO of neutral 10-azaBaP. (c) HOMO of neutral 4-azaBaP.

5-epoxide, followed by *trans* and *cis* opening of the epoxide, have been reported.[13] These adducts were calculated in the present work in order to examine their structures and relative energies. The most stable stereoisomer (by less than 2 kcal mol^{-1}) was the one with the cytosine moiety *trans* to the hydroxyl, with both groups in pseudoequatorial conformation (as was inferred in ref. 13). Two structures, differing in energy by less than 1 kcal mol^{-1}, were characterized for the *cis* isomer, each presenting one group in pseudoequatorial conformation while the other group was pseudoaxial. The most stable *cis* isomer was the one with the cytosine in the pseudoaxial disposition (OH pseudoequatorial). Based on spectral studies,[13] it was proposed that the adduct had a pseudoequatorial–pseudoaxial conformation, although the cytosine residue was supposed to be pseudoequatorial. The relative energies are consistent with the strength (bond distance) of the hydrogen bond generated between the hydroxyl and the cytosine residue, which were 2.010, 2.280 and 2.313 Å, for the *trans*, *cis* (OH pseudoequatorial), and *cis* (cytosine pseudoequatorial) isomers, respectively. However, taking into account the similar energies of these structures, it is anticipated that comparable amounts of each isomer would be formed. The computed adducts are displayed in Fig. 6.

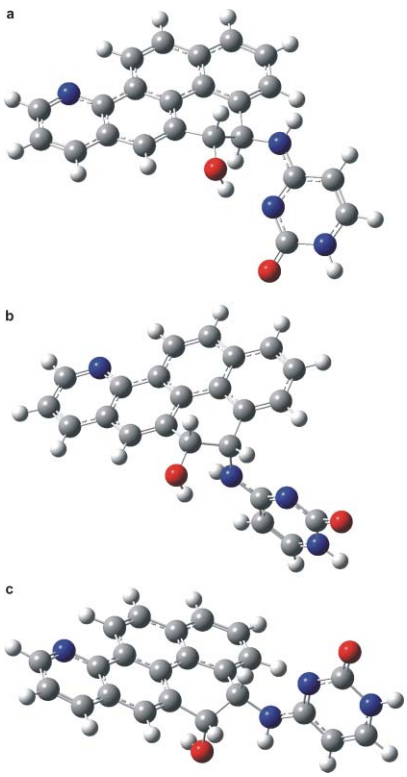


Fig. 6 Lowest energy conformations of the adducts of 10-azaBaP-4,5-epoxide with cytosine. (a) *Trans* conformation. (b) *Cis* conformation (OH pseudoequatorial). (c) *Cis* conformation (cytosine residue pseudoequatorial).

Concluding remarks

The DFT calculations in the present model study regarding the relative stabilities of the carbocations generated from oxidized metabolites of BaP, BeP, and azaBaPs appear to correlate with the available literature data on the biological activities of their diol epoxides. Benzylic carbocations derived from BaP are more stable than those derived from BeP and azaBaPs. For the azaBaPs studied, the predicted reactivity order is 6-azaBaP > 4-azaBaP > 10-azaBaP, which is attributed to the degree of delocalization of the net positive charge throughout the aromatic system, as indicated by the development of negative charge density at the carbocationic center. The magnitude of the negative charge at nitrogen was also important for carbocation stability. On the other hand, protonation reactions for the azaBaP derivatives appear to be governed by the HOMO of the neutral compounds. Calculations of the cytosine adducts, resulting from the *trans* and *cis* openings of the epoxide of 10-azaBaP-4,5-epoxide, suggest that both isomers should form in nearly equal amounts experimentally.

Fluorination led to a decrease in carbocation stability. This decrease was less pronounced for fluorine substitution at the highly positively charged sites due to p–π back-bonding and fluoronium character development. On the other hand, F-substitution at a position with negative charge density produced a greater reduction in the exothermicity of the ring opening reaction. This was a general trend observed in the absence of any other interactions, such as attractive F · · · H interactions and repulsive/steric interactions between F and an OH group.

Synthesis and DNA binding studies of the fluorinated derivatives, for which biological activity assessments are still not available, will allow a more comprehensive evaluation of the relative reactivities that are predicted by theoretical results in this work.

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