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Carbocations from Oxidized Metabolites of Benzo[a]anthracene. A Computational Study of Their Methylated and Fluorinated Derivatives and Guanine Adducts

Gabriela L. Borosky[†] and Kenneth K. Laali^{*,‡}

[†] *Unidad de Matemática y Física, INFIQC, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba 5000, Argentina*

[‡] *Department of Chemistry, Kent State University, Kent, Ohio 44242, USA*

Abstract

Structure-reactivity relationships and substituent effects on carbocation stability in benzo[a]anthracene (BA) derivatives have been studied computationally at the B3LYP/6-31G* and MP2/6-31G** levels. Bay-region carbocations are formed by O-protonation of the 1,2-epoxides in barrierless processes. This process is energetically more favored as compared to carbocation generation via zwitterion formation/O-protonation, via single electron oxidation to generate a radical cation, or via benzylic hydroxylation. Relative carbocation stabilities were determined in the gas phase and in water as solvent (PCM method). Charge delocalization mode in the BA carbocation framework was deduced from NPA-derived changes in charges, and substitution by methyl or fluorine was studied at different positions selected on basis of the carbocation charge density. A bay-region methyl group produces structural distortion with consequent deviation from planarity of the aromatic system, which destabilizes the epoxide, favoring ring opening. Whereas fluorine substitution at sites bearing significant positive charge leads to carbocation stabilization by fluorine p- π back-bonding, a fluorine atom at a ring position which presented negative charge density leads to inductive destabilization. Methylated derivatives are less sensitive to substituent effects as compared to the fluorinated analogues. Although the solvent decreases the exothermicity of the epoxide ring opening reactions due to greater stabilization of the reactants, it provokes no changes in relative reactivities. Relative energies in the resulting bay-region carbocations are examined taking into account the available biological activity data on these compounds. In selected cases, quenching of bay-region carbocations was investigated by analyzing relative energies (in the gas phase and in water) and geometries of their guanine adducts formed via covalent bond formation with the exocyclic amino group and with the N-7.

Introduction

Mutagenic and/or carcinogenic polycyclic aromatic hydrocarbons (PAHs)¹ are widespread environmental pollutants (1). The main metabolic pathway for activation of these compounds involves formation of bay-region diol epoxides (DEs) (2). Benzylic carbocations generated from these electrophilic DEs by opening of the epoxide ring are capable of forming covalent adducts with the nucleophilic sites in DNA and RNA, leading to alteration of genetic material

* To whom correspondence should be addressed. Tel: 330-6722988. Fax: 330-6723816. E-mail: KLaali@kent.edu.

¹Abbreviations: PAH, polycyclic aromatic hydrocarbon; DE, diol epoxide; BA, benzo[a]anthracene; 7-MBA, 7-methylbenzo[a]anthracene; 7,12-DMBA, 7,12-dimethylbenzo[a]anthracene; DFT, density functional theory; NPA, natural population analysis; NBO, natural bonding orbital; PCM, polarized continuum model; RC, radical cation; ZPE, zero-point energy; MP2, second-order Møller-Plesset perturbation theory.

(3,2). Adduct formation is accepted as a critical step in the mechanism by which PAHs can cause mutations resulting in induction of cancer (2).

The carcinogenic activity of polyarenes is frequently enhanced by methyl substitution at strategic positions. In this way, methyl substitution at a bay region tends to markedly increase the biological activity (4). Among benzo[*a*]anthracenes (BAs), the unsubstituted parent compound presents borderline activity (5), but 7-methylbenzo[*a*] anthracene (7-MBA) is considerably more active (it is the strongest tumor initiator among the twelve possible monomethylated BAs, followed by the 6-CH₃, 8-CH₃, and 12-CH₃ derivatives) (6–9), while 7,12-dimethylbenzo[*a*]anthracene (7,12-DMBA) is one of the most potent carcinogens known (10). On the other hand, low tumorigenicity has been observed for 1-, 2-, 3- and 4-MBA (6). Among other dimethyl-BA compounds, the 6,8-dimethyl- and 8,9-dimethyl-BA have also been reported to have relatively high activity (4).

For 7,12-DMBA not only DE formation, but also the radical cation process (via one-electron oxidation) and benzylic sulfate ester formation (via initial formation of benzyl alcohol) could contribute to its metabolic activation (11). For the benzylic-DNA adducts, the 12-CH₂OH and 7-CH₂OH could both be involved in adduct formation (12).

Carcinogenic activity of PAHs is often strongly affected by fluorine substitution at strategic molecular sites (2,13). Whereas fluorine substitution at C-10 in 7-MBA and 7,12-DMBA led to markedly enhanced tumor initiating activity in mouse skin and mutagenic activity in human hepatoma (HepG2) cell-mediated assay (14–16), F-substitution at C-1, C-2, C-3, C-4, and C-5 resulted in lower bioactivity relative to 7,12-DMBA (15,17–19). For other regioisomeric fluoro derivatives of 7,12-DMBA, there was no significant change in activity (14,17). Similar F-substitution effects were also observed for the 7- and 12-monomethyl analogues of BA (16, 20–22). Effect of a fluoro substituent at C-9 and C-10 on tumorigenicity of 7-MBA-3,4-diol, 12-MBA-3,4-diol, and 7,12-DMBA-3,4-diol was also studied (21). It was argued that F-substitution at C-10 in BA increases the metabolic formation of the 3,4-diol, with no significant effect on the reactivity of the bay-region anti-DE (21). The 1,2,3,4-tetrahydro-7,12-DMBA (THDMBA) is an animal carcinogen without an aromatic bay-region. Its 6-F-derivative is twice as potent as the parent compound, whereas the 5-F-derivative is inactive (19). Study of their metabolites indicated that benzylic hydroxylation at C1 and side chain hydroxylation at 12-Me are important in the metabolism of the reactive 6-F-derivative (23).

Very good agreement with the experimental reactivities of several PAH derivatives have been shown by recent quantum-mechanical calculations when applied to the study of the carcinogenic pathways of these compounds (24–28). Besides, modeling studies of biological electrophiles from PAHs by DFT methods have yielded appropriate descriptions of the NMR features and charge delocalization modes of their resulting carbocations (29–39).

In this work we have performed a model DFT study on the structural and electronic properties of the reactive intermediates of BA derivatives, starting with the bay-region epoxides. As in previous computational studies (24–28), these intermediates have proven to generate the same reactivity trends as their DE analogs, while requiring considerably less computational time. The present study was inspired by our earlier stable ion work on the BA carbocations (31) and sets the stage for a more comprehensive structure/reactivity study with a larger set of regioisomeric mono- and dialkylated BAs. Fluoro and methyl substitution at selected molecular sites was investigated. Changes in energy for epoxide ring opening reactions were calculated in gas phase and in water as solvent. 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).

To model the crucial step of covalent adduct formation, adducts resulting from quenching of selected carbocations (derived from their corresponding epoxides) with guanine via the exocyclic amino group and via the N-7 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 (40), employing the B3LYP (41–43) functional and the 6-31G* split-valence shell basis set. 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 (44). The solvent effect was estimated by the polarized continuum model (PCM) (45–48) without geometry optimizations unless specified. Zero-point energy (ZPE) corrections were included for gas-phase results. MP2 single point energy calculations were performed with the 6-311G** basis set starting with the B3LYP/6-31G* optimized geometries (MP2/6-311G**//B3LYP/6-31G*). AMI semiempirical calculations (49) were utilized for initial conformational searches in the covalent adducts and the most favored conformations were selected for energy calculations by DFT.

Results and Discussion

Mechanistic studies on the hydrolysis of benzo[*a*]pyrene-DE demonstrated pH dependency and catalysis by DNA and by polynucleotides, showing that protonation must occur before or during the rate determining step (50,51). Based on these studies, it was proposed that a physically-bound DE reacts to form a physically bound benzylic carbocation in the rate determining step. The monohydrogen phosphate group on the nucleotide acts 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 (52–54). Taking this into account, formation of the benzylic carbocation derived from BA-1,2-epoxide was calculated by means of two possible pathways: by epoxide ring opening of the O-protonated species, and via a mechanism involving zwitterion formation by heterolytic opening of the neutral epoxide and subsequent protonation (Figure 1). Comparison of the changes in energy for both paths served as a test for selecting only the most likely process for the entire study. The same calculations were performed for 12-MBA in order to account for the presence of a bay-region methyl group.

For both zwitterion calculations the position of the hydrogen atom attached to C-2 could not be fully optimized because, if its bond length to C-2 was not constrained, it migrated spontaneously to generate the more stable 2-keto compound. Conversely, the corresponding protonated epoxides, *i.e.*, the oxonium ions, could not be located as minima on the potential energy surface, as in both cases the epoxide ring opened by a barrierless process upon O-protonation. Zwitterion formation was endothermic by *ca.* 40 kcal/mol, while both protonation steps were highly exothermic. Consequently, the protonated epoxide → carbocation pathway was selected for computations involving the rest of the compounds in the present study, since it was established as the most favored mechanism.

The charge delocalization map for BA is shown in Figure 2. According to the NPA-derived charge distribution, positive charge in the resulting carbocation is delocalized throughout the π -system. Earlier stable ion NMR studies have shown that arenium ions and benzylic carbocations derived from BA exhibit strong anthracenium ion character (31).

Regarding the charge delocalization mode for BA, substitution at positions 6 and 3 of the 1,2-epoxide would be expected to produce the most noticeable effects, followed by positions 7 and 10. On the other hand, substitution at positions 4 and 5 would present the strongest effects in the opposite sense, as these sites develop negative charge density upon carbocation formation. Bearing this in mind, calculations for the protonated-epoxide opening reaction (reaction 1 type) were carried out for eight monomethylated derivatives of BA-1,2-epoxide.

(1).

Positions 4 and 3 were left unsubstituted since these sites give rise to the 3,4-diol, which is then epoxidized to the bay-region DE, in line with the low tumorigenicity caused by methyl substitution at the 1-, 2-, 3-, 4-positions (6). The dimethylated compound 7,12-DMBA was also considered. Calculation results are collected in Table 1. Changes in NPA-derived charges are included for the carbocation centre and the methyl group (carbon plus attached-hydrogen charges). It should be noticed that the same observation regarding the instability of the oxonium ion was made for every protonated epoxide in the present study.

The ΔE_r for the opening reactions could be viewed as a measure of the stability of the resulting carbocations. In relation to this, good correlations have been obtained between the calculated relative energies for carbocation formation from various PAHs and their reactivity towards nucleic acid (24–28). Moreover, a clear relationship was observed between the extent of charge delocalization in the carbocation (assessed by the decrease in positive charge at the carbocationic centre within series of compounds) and the ease of its formation (24–26).

The present calculated results in Table 1 show a general agreement with the known carcinogenic activities of the BA series, even though differences in energy are small. It should be noted that no clear correlation had been observed between mutagenicity and carcinogenic potency for BA, 7,12-DMBA, and the twelve isomeric MBAs (55). In this way, while 7,12-DMBA was the most potent mutagen, followed by 7-MBA, the moderately strong and weak carcinogens were not distinguishable by their bacterial mutagenicity, which differed depending on the experimental conditions (55). Nevertheless, these theoretical computations follow the carcinogenic trend of this type of compounds fairly well, given that no conclusive experimental activity order is available, and considering that in the calculations the biological environment effects (in particular differences in DNA repair) are not taken into account. Thus, according to ΔE_r , carbocation formation from 7,12-DMBA is the most favored reaction. The isomeric monomethylated derivatives all exhibited similar ΔE_r values, with 12- and 6-MBA being among the most reactive ones, while 11- and 5-MBA corresponded to the least exothermic reactions, as it was expected. Among the monomethyl epoxides, 12-MBA yielded the most favorable ΔE_r . Since parent BA was least reactive in the series, methyl substitution resulted in stabilization of the carbocations. As no significant hyperconjugative effects were assessed according to NBO analysis (no depletion of the C-H bonds of the methyl group), these observations indicate that the methyl group causes inductive stabilization. The MP2/6-311G** energies showed the same reactivity tendencies as the B3LYP results.

12-MBA and 7,12-DMBA, *i.e.*, both bay-region methylated compounds, present a deviation from planarity of the aromatic system at the bay region due to steric strain induced by the methyl group. The enhanced carcinogenic activity induced by methyl substitution at the bay region has been explained by a more favored opening of the epoxide ring (24). This fact stems

from instability of the ring closed species, which presents a distorted non-planar structure (56), rather than from stabilization of the open carbocation by methyl substitution.

Another major activation pathway that has been invoked in metabolism of 7,12-DMBA is the radical cation (RC) mechanism (57–60). This was taken into account in the present study by considering the formation of the RC of 7,12-DMBA (reaction 2). This species presented both charge and spin densities mainly located at C-7 and C-12 (Figure 3), with negligible hyperconjugative stabilization by the methyl substituents.

(2).

Additionally, the *meso*-region theory proposes the formation of *meso*-hydroxymethyl metabolites, which subsequently generate benzylic-DNA adducts (61–65). In case of 7–12-DMBA, involvement of the 12-CH₂OH and 7-CH₂OH have been invoked in adduct formation via this pathway, with hydroxylation suggested as the rate-limiting step (72). Accordingly, formation of the 7-CH₂⁺ and 12-CH₂⁺ carbocations from their corresponding alcohols were calculated in order to take into consideration this metabolic mechanism (Figure 4).

Hence, the change in energy for reaction 2 and for the hydroxymethyl paths in Fig. 4 was analyzed and compared with the thermodynamics of the epoxidation process (reaction 3).

(3).

With this approach, ΔE_r for reaction 2 was 149.49 kcal/mol (115.33 kcal/mol in water as solvent). On the other hand, hydroxylation followed by formation of the 7-CH₂⁺ and 12-CH₂⁺ carbocations were exothermic by *ca.* -87 kcal/mol (-94 kcal/mol in water) and -218 kcal/mol (-146 kcal/mol), respectively. Moreover, in the framework of the *meso*-region theory, one-electron oxidation of 7,12-DMBA coupled with oxygen transfer has been reported to yield both 7- and 12-hydroxymethyl isomers (66), which determines that primary benzylic alcohol formation would also be dependent on the energetic of reaction 2. For reaction 3, ΔE_r was -58.30 kcal/mol (-62.19 kcal/mol in water), with subsequent epoxide opening reaction 1 for 7,12-DMBA-1,2-epoxide being highly exothermic (-238.09 kcal/mol, -170.64 kcal/mol in water). Therefore, these computations point to the DE mechanism as the energetically more favored metabolic path for 7,12-DMBA.

Turning our attention to the fluorinated analogs, calculations were carried out for the 5-, 6-, 9-, 10- and 12-fluorinated derivatives of 7-MBA. Fluorination at C-12 was analyzed in order to reveal any influence that may be exerted by the fluorine atom when located in a bay region, and the 7-FBA was considered for comparison with 7-MBA. Results are gathered in Table 2.

By taking into account the $\Delta E_r S$ for epoxide ring opening of the fluorinated compounds, it is noted that the 6-F structure is most favored for opening of the 1,2-epoxide, yielding a ΔE_r even more exothermic than the unsubstituted molecule. Hence, a fluorine atom at a highly positively charged site stabilizes the carbocation. The 10-F derivative was less reactive than nonfluorinated 7-MBA, this fact being in contrast with the experimental tumorigenic activities. However, subsequent studies have suggested that the influence of fluorine at C-10 of BA is not due to increased reactivity of the bay-region DE but rather likely on the initial formation of the 3,4-diol (21). Both 10-F-7-MBA and 7-MBA afforded ΔE_r s more exothermic than 9-F-7-MBA, in accord with the biological activity of these derivatives (16,20–22). On the other hand, F-substitution at a position with negative charge density (C-5) decreased the exothermicity of the opening reaction, while 7-FBA also gave a less favored value than 7-MBA, both results being in agreement with their reported relative tumorigenicities (23,22).

In addition, a correlation was found between exothermicity of the epoxide ring opening and the C-F bond length, in the sense that the C-F bond becomes shorter with increasing stability of the carbocation. This observation corresponds to electron density loss at F, assessed by NPA-derived charges. Hence, fluorine stabilization of the PAH carbocation correlates with the degree of fluoronium ion character, which overrides the unfavorable inductive electron withdrawal of F. Fluorine substitution was previously seen to have a major impact on the charge distribution of PAH carbocations (67,68) and dications (68). The exception to the observations made above was the 12-F compound. In this case a hydrogen bond interaction takes place between fluorine and the hydrogen atom attached to C-1. This interaction is stronger in the open carbocation (H-F distance is 2.0376 Å in the carbocation vs. 2.0633 Å in the epoxide), thus favoring epoxide ring opening, which agrees with the reported activity (22). In general, larger variations in ΔE_r among the different fluoro analogues were observed, indicating that fluoronium ion character has a stronger influence than the inductive effect exerted by the methyl group in the methylated derivatives.

Aqueous-phase calculations (PCM method with water as solvent) afforded the same reactivity patterns as those in the gas phase, when comparing any given series of compounds/reactions, *i.e.*, inclusion of the solvent did not alter the relative reactivity trends. However, despite the greater stabilization of the carbocations as compared to the neutral epoxides, the ring opening reactions were less exothermic in water owing to the large solvation energy of proton.

Guanine covalent adducts

It has been established that bay-region DEs derived from PAHs predominantly form stable adducts with DNA by *cis* or *trans* addition of the exocyclic amino groups of deoxyguanosine and deoxyadenosine (69). Moreover, depurinating products that are lost from DNA by cleavage of the glycosidic bond are formed by reaction with the N-7 of guanine, and at N-3 and N-7 of adenine (70). Bearing this in mind, the adducts arising from reactions with the exocyclic nitrogen and with the N-7 of guanine were calculated for the carbocations derived from 7,12-DMBA-1,2-epoxide and 12-F-7-MBA-1,2-epoxide. 7,12-DMBA was selected considering its strong carcinogenic potency, and the 12-F derivative was chosen as model to examine the influence of a bay-region fluorine atom. The major 7,12-DMBA adduct formed *in vivo* corresponds to the one derived from the *anti*-DE with deoxyguanosine (71). The *trans* products were considered, this mode of attack being preferred for *anti*-DEs (72). Conformational searches by rotation of the generated C-N bond were performed by AM1 calculations in order to find the most stable rotamer for each compound. These lowest energy structures were subsequently selected for optimization by DFT calculations (Table 3, Figures 5 and 6). Superimposed structures are shown in Figure 7.

For 7,12-DMBA-1,2-epoxide, a perpendicular arrangement between the two aromatic moieties was the most favored disposition for both adducts. A noteworthy feature in this adduct is

deviation from planarity in the system which is caused by steric interaction between guanine and the 12-Me group. According to the gas-phase calculations, the most stable isomer was the N-7 product, *ca.* 1 kcal/mol lower in energy than the exocyclic adduct. This fact is probably due to the stabilization brought about by two intramolecular hydrogen bonds in the N-7 product (between the guanine carbonyl oxygen, the bay-region hydrogen, and one of the 12-Me hydrogens), *vs.* one hydrogen bond in the exocyclic adduct. However, aqueous-phase energy computations reverses the relative stability of these adducts, with the exocyclic one becoming more stable by over 2 kcal/mol.

In the case of 12-F-7-MBA-1,2-epoxide, calculations in the gas phase showed that the N-7 adduct is once again more favored (by about 1 kcal/mol), with one hydrogen-bond interaction being attained between the guanine carbonyl and the bay-region hydrogen. In the exocyclic adduct, the aromatic moieties lose coplanarity in order to form a hydrogen bond between the fluorine and the hydrogen atom attached to N-1. Interestingly, computations in water (both single points and optimizations) afforded almost the same energy for both adducts, suggesting the formation of near equal amounts of the exocyclic and N-7 adducts.

Reaction energies for the formation of each type of adduct with both carbocations (measured as the energy difference between the adduct plus proton, minus guanine and carbocation total energies) were comparable, although slightly more favored for 12-F-7-MBA-1,2-epoxide. Hence, the presence of a fluorine atom at the bay region seems to increase reactivity as compared to methyl substitution at the same position. Inclusion of the solvent led to a decrease in the endothermicity of the reactions as a result of better proton solvation.

Benzylic 12-CH₂-adducts from 7,12-DMBA are originated by both the RC and *meso*-region pathways. In order to compare their relative stabilities with those derived from the DE path, the 12-CH₂-exocyclic adduct was computed. Calculations afforded a ΔE_r for formation comparable with those found for the adducts from 7,12-DMBA-1,2-epoxide (Table 3). According to these results, the relative amounts of adducts arising from covalent binding to DNA by means of alternative metabolic mechanisms would be primarily determined by the plausibility of generation of the respective ultimate electrophilic PAH metabolites.

Concluding remarks

According to the present DFT study, stability of the carbocations generated from oxidized metabolites of B A seems to correlate with the available data on their biological activity. Calculations in this work point to the DE mechanism as the main metabolic activation pathway for BA and its methylated and fluorinated derivatives. Epoxide protonation/ring opening is strongly preferred over zwitterion formation/protonation. The MP2/6-311G** relative reactivities agreed with the B3LYP/6-31G* results.

Methylated compounds exhibit more exothermic ΔE_r s than the nonmethylated analogue, although very similar values were obtained for the methylated series. As NBO analysis did not reveal significant hyperconjugation effects, it is inferred that methyl substitution stabilizes the carbocations mainly by donor inductive effect. The neutral epoxide presented deviation from planarity in the aromatic system when a methyl group is substituted at C-12. Therefore, enhanced carcinogenic activity caused by methyl substitution at the bay region is explained by preferential opening of the epoxide ring due to higher energy of the more crowded epoxide.

A fluorine atom at a highly positive charged site stabilizes the carbocations by p- π back-bonding and development of fluoronium ion character, which overrides the inductive electron withdrawal of F. Correlation between stability of the generated carbocation and the loss in electron density at F corresponds with shortening of the C-F bond length. Among the fluorinated compounds, the 6-F structure is best for opening of the 1,2-epoxide, affording a

ΔE_r more exothermic than the nonfluoro structure. On the other hand, F-substitution at a position bearing negative charge density decreases the exothermicity of the ring opening reaction. In the case of the 12-F compound a hydrogen bond interaction takes place between fluorine and the hydrogen atom attached to C-1. This interaction assists the ring opening reaction as it is stronger in the open carbocation than in the epoxide. In general, fluorine exerted a stronger influence than methyl, as F-substitution at different sites generated larger variations in the ring opening reaction energy as compared to the methylated compounds.

Aqueous-phase calculations yielded the same reactivity as gas-phase computations, although epoxide ring opening was less exothermic in water because of the large solvation energy of the reactants. The change in the preferred product of the addition reactions with guanine due to solvent effect is noteworthy for 7,12-DMBA-1,2-epoxide. Whereas the N-7 adduct was most stable in gas phase, in water as solvent the most stable adduct was the one formed by reaction with the exocyclic nitrogen of guanine, in accordance with experimental observations, suggesting that DFT studies on the reactivity of this type of compounds could provide reasonable estimates when extrapolated to living organisms. On the other hand, for the less studied 12-F-7-MBA derivative, the present study points to the formation of both type of guanine adducts in near equal amounts. This finding must await further experimental verification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.

Alternative mechanistic pathways for activation of benzo[*a*]anthracene-1,2-epoxide and 12-methyl-benzo[*a*]anthracene-1,2-epoxide (aqueous-phase values in parenthesis).

Figure 2.

Computed gas-phase NPA heavy atom charge densities (Δ charges relative to the neutral compound in parentheses) for the carbocation generated from BA-1,2-epoxide. [Dark circles are roughly proportional to the magnitude of C Δ charges; threshold was set to 0.030].

a

b

Figure 3. 7,12-DMBA radical cation; (a) computed gas-phase NPA heavy atom charge densities (Δ charges relative to the neutral compound in parentheses; ^acarbon plus hydrogens); [Dark circles are roughly proportional to the magnitude of C Δ charges; threshold was set to 0.030]; (b) spin density.

Figure 4. Generation of benzylic 7- CH_2^+ and 12- CH_2^+ carbocations from 7,12-DMBA (aqueous-phase values in parenthesis).

Figure 5. Adducts formed between guanine and 7,12-DMBA-1,2-epoxide; (a) exocyclic N; (b) N-7 isomer.

Figure 6. Adducts formed between guanine and 12-F-7-MBA-1,2-epoxide; (a) exocyclic N; (b) N-7 isomer.

Figure 7. Superimposed structures of the adducts of 7,12-DMBA-1,2-epoxide and 12-F-7-MBA-1,2-epoxide with guanine; (a) exocyclic N-adduct; (b) N-7 adduct.

Table 1

Calculations for the Methylated Derivatives of BA-1,2-Epoxyde

Methylated position	ΔE_r (kcal/mol)		Δq^a	$\Delta qC-1^b$	ΔqCH_3^c	Carcinogenic activity ^d
	B3LYP/6-31G*	MP2/6-311G** ^e				
	Gas phase ^f (Aqueous phase) ^g	Gas phase				
7,12	-238.09 (-170.90)	-233.78		-0.102	0.045 (7-Me) 0.024 (12-Me)	Very potent
7	-235.01 (-168.95)	-230.92	0.078	-0.088	0.042	Potent
12	-236.12 (-170.47)	-232.34	0.027	-0.096	0.022	Moderately active
6	-235.81 (-169.18)	-232.05	0.147	-0.059	0.059	Moderately active
8	-234.46 (-168.77)	-230.35	0.031	-0.085	0.032	Moderately active
10	-235.27 (-169.21)	-231.16	0.060	-0.086	0.041	Weakly active
9	-235.27 (-168.84)	-231.86	0.020	-0.084	0.035	Weakly active
5	-234.41 (-168.42)	-231.28	-0.035	-0.086	0.041	Weakly active
11	-233.87 (-168.28)	-230.26	0.000	-0.084	0.025	Weakly active
BA	-233.29 (-168.72)	-229.63	-	-0.081	-	Weakly active

^aChange in charge density for the indicated carbon atom in the nonmethylated compound (qCcarbocation – qCepoxide).

^bChange in charge density at the carbocation center, between the open carbocation and the neutral closed epoxide (qC-1 carbocation – qC-1 epoxide).

^cChange in charge density between the open carbocation and the neutral closed epoxide (qCH₃carbocation – qCH₃epoxide).

^dAs compiled in (55).

^eSingle-point energy calculation.

^fZPE correction included.

^gPCM optimizations.

Table 2
Calculations for the Fluorinated Derivatives of 7-MBA-1,2-Epoxyde

Fluorinated position	ΔE_r (kcal/mol)		Δq^a	Δq_{C-1}^b	Δq_F^c	C-F bond length (Angstroms)
	ΔE_r (kcal/mol)					
	B3LYP/6-31G*	MP2/6-311G** ^d				
	Gas phase ^e (Aqueous phase)					
	Gas phase					
6	-235.94 (-170.02)	-232.20	0.147	-0.093	0.051	1.3261
12	-235.58 (-168.84)	-231.42	0.027	-0.086	0.018	1.3485 ^f
10	-233.90 (-168.25)	-229.18	0.060	-0.086	0.033	1.3324
9	-232.72 (-167.62)	-228.80	0.020	-0.084	0.027	1.3365
5	-230.53 (-164.21)	-225.96	-0.035	-0.063	0.026	1.3413
7-F-BA	-231.94 (-167.20)	-227.67	0.078	-0.086	0.033	1.3360

^aChange in charge density for the indicated carbon atom in BA ($q_{Ccarbocation} - q_{Cepoxide}$).

^bChange in charge density at the carbocation center, between the open carbocation and the neutral closed epoxide ($q_{C-1carbocation} - q_{C-1epoxide}$).

^cChange in charge density between the open carbocation and the neutral closed epoxide ($q_{Fcarbocation} - q_{Fepoxide}$).

^dSingle-point energy calculation.

^eZPE correction included.

^fThe fluorine atom forms a hydrogen-bond interaction.

Table 3
Calculations for Adducts of 1,2-Substituted-7-MBA-1,2-Epoxyde with Guanine

Carbocation	Reacting Nitrogen of Guanine	Total Energy (hartree)		Relative Energy (kcal/mol)		$\Delta E_{\text{reaction}}$ (kcal/mol) ^a	
		Gas phase	Aqueous phase	Gas phase ^b	Aqueous phase	Gas phase ^b	Aqueous phase
1,2-CH ₃	Exocyclic	-1389.52582	-1389.57655	1.13	0.00	222.81	155.04
	N-7	-1389.52800	-1389.57260	0.00	2.48	221.68	157.52
	Exocyclic ^c	-1313.13108	-1313.17085	-	-	220.80	153.73
1,2-F	Exocyclic	-1449.45704	-1449.50303	1.58	2.21	218.78	154.60
			(-1449.50846) ^d		(0.12)		
	N-7	-1449.45912	-1449.50655	0.00	0.00	217.20	152.39
			(-1449.50865) ^d		(0.00)		

^a $\Delta E_{\text{reaction}} = \text{EnergyAdduct} + \text{EnergyH}^+ - \text{EnergyGuanine} - \text{EnergyCarbocation}$.

^b ZPE correction included.

^c Adduct from 7,1,2-DMBA.

^d PCM optimization.