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Carcinogenicity of benzo[*a*]pyrene diol epoxide stereoisomers: A linear free energy relationship study

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Abstract

In this work, we use linear free energy relationships as a comparative tool to study chemical reactivity of the four stereoisomers of the ultimate carcinogen benzo[*a*]pyrene 7,8-diol-9,10-epoxide at *N*-cyclic or *N*-exocyclic positions of guanine and adenine. Calculations were performed with PM3 semiempirical method and by applying density functional theory at B3LYP/6-31G* level of theory. Reaction free energies were obtained with water environment modeled by either the dielectric continuum method of Tomasi and co-workers, or the discrete Langevin dipoles model of Florián and Warshel. Except for reactions occurring at N^2 site of guanine, good correlations with literature data were found, highlighting in particular the (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene molecule often reported as being the most tumorigenic of the four isomers. The possibility of an anchimeric assistance mechanism for this specific stereomer is discussed. Finally, an interesting relationship between hydration free energies and the total dipole moment of the adducts was evidenced, suggesting a possible solvent effect to play a particularly important role in the reactivity of these polyaromatic hydrocarbons toward N7 position of adenine.
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Keywords: Cancer; Carcinogenesis; Ultimate carcinogens; Benzo[*a*]pyrene diol epoxide (BPDE) stereoisomers; Density-functional calculations (DFT); Linear free-energy relationships (LFER); Reactivity; Solvent effects

1. Introduction

The most common cancer in the world today is lung cancer, accounting for 18% of cancers of men worldwide, and 21% of cancers in men in the developed countries [1], and tobacco has been clearly shown to be the major risk factor in broncho-pulmonary oncogenesis. Bay region benzo[*a*]pyrene 7,8-diol-9,10-epoxides (BPDE) are formed through the metabolism of benzo[*a*]pyrene (BP) [2], an ubiquitous environmental carcinogen, present in vehicle exhausts, smoke- or heat-processed food, and cigarette smoke [3,4]. The metabolic activation of this polycyclic aromatic hydrocarbon (PAH) to BPDE, its ultimate carcinogenic metabolite, is catalyzed by liver microsomal enzymes (P-450 family, epoxide hydrolase, prostaglandin H synthase) and successively implies the BP-7,8-oxide and BP-7,8-dihydrodiol intermediates. BPDE will react with purine DNA bases through a S_N1 or S_N2 mechanism. It may also form adducts with the ribose moiety of adenosine [5] or more generally with RNA, proteins [6], e.g. human serum albumin [7], or other biomolecules.

Even though reaction of aralkylating agents like BPDE, in contrast to alkylating agents, mostly takes place at exocyclic amino groups of deoxyadenosine and deoxyguanosine, minor adducts at the amino group of deoxycytidine, as well as at N7 of guanine have been observed in some cases, the latter being unstable and resulting in depurination [8]. Nevertheless, as Cheng and co-workers mention [9], even minor adducts may be biologically important and potentially able to activate an oncogene. To our knowledge, products at N7 of adenine have not been reported yet.

In the metabolic pathway of BP to BPDE, the arene oxides, among other reactions, undergo hydration catalyzed by epoxide hydrolase to the corresponding *trans*-dihydrodiols. In this way, four optically active isomers may exist, where each diastereomeric BPDE (7-OH either *cis* or *trans* to the 9,10-epoxide) can be resolved into two enantiomers of opposite handedness [10]. Depending upon in vitro (microsomal systems) or in vivo (cellular models or animals) observations, extents of formed BPDE enantiomers vary; in humans, the (7*S*,8*R*,9*R*,10*S*)-BPDE (BPDEαββ) form has not been demonstrated yet to be metabolized from BP [7]. It is also

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not obvious to conclude from the presence or not of a specific DNA adduct to what extent the corresponding enantiomer has been synthesized. As all four configurational isomers were detected in vitro, it is plausible that they might as well be formed in vivo [6,11]. Several animal models were studied to investigate the mutagenic and carcinogenic abilities of the four optically pure synthetic isomers of BPDE, as well as various bacteria strains as for their mutagenic effects [12]. (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDEβααα) was generally found to be the most mutagenic (with the exception of bacteria) and carcinogenic form of BPDE. For example, in newborn mice [10], only this diol epoxide showed exceptional tumorigenicity, the others having little or no activity.

Each (+) or (−) enantiomer can react via *cis* or *trans* addition at the benzylic C10 position with the exocyclic amines of guanine and adenine [13], leading to potentially 16 total possibilities of stereoisomeric adducts. However, it has been observed that for dihydrodiol epoxides derived from planar hydrocarbons, the amino group of deoxyguanosine residues in DNA is the preferential site of reaction [6,14], with exceptions such as the fjord-region dibenzo[*a*,*l*]pyrene diol epoxides, binding preferentially on deoxyadenosine [15]. More specifically, the (±) *anti*-dihydrodiol epoxide opened *trans* by nucleophilic attack on the oxirane of the N^2 amino group of guanine seems to be the major reaction occurring in vivo [9] (Fig. 1), whereas for (±) *cis*-dihydrodiol epoxide, the opening has been shown to be rather *cis* [5].

Once the reaction has proceeded with one or the other isomer, the question of the conformation and stability of the BPDE–DNA adduct at its DNA location arises, and it is believed to be relatively stable in general [14]. This aspect has been extensively studied by Geacintov and co-workers [13,16–19]. They have shown for instance that the 10*S* (+)-*trans*–*anti*-[BP]-N^6-dA (AEβααβ) is significantly more exposed to the solvent as is its enantiomer 10*R* (−) (AEαββα), which is an intercalative adduct [13]. This may explain greater susceptibility of the first to removal by human nucleotide excision repair enzymes, and account for differences in mutagenicity. Mutation induction can indeed occur through stable adduct formation, and most PAH diol epoxide studies have been consistent with this mechanism, but unstable adducts that depurinate to leave apurinic mutagenic sites also occur [15,20–22].

Finally, it is to notice that other structural factors influence chemical reactivity of BPDE toward DNA, e.g. (i) the formation of a benzylic carbocation intermediate can be catalyzed by DNA itself [8,23–25], and secondly, hydrogen-bonded base pairs function as a general base catalyst, thus increasing the nucleophilicity of the amino groups [26,27], (ii) methylation of cytosines in CpG dinucleotides, for instance in P53 codons which are known to be the major mutational hot spots in lung cancer, has been proven to drastically enhance BPDE adduct formation [28–30].

Given the still incomplete and often not easily comparable data in the current literature, notably because one set of properties obtained for a given animal and/or cell/tissue target is not necessarily transposable to another (and a fortiori for humans), we thought that it would be useful to try to rationalize the BPDE/guanine or adenine reaction problem under the point of view of chemical reactivity. In this respect, we chose in our present paper the simple approach consisting in applying the linear free energy relationships (LFER), an established method for comparing activation free energies. Indeed, comparison of these energies extrapolated from the calculated free energies of reaction is appropriate due to the fact that, within each guanine or adenine set of reactions, BPDE stereoisomeric reactants as well as the corresponding product stereoisomers are very structurally close. After first optimization in vacuo at the PM3 semiempirical level, we performed the computations of free energies of reactions (ΔG_{reac}) at the B3LYP DFT level of theory, using as a model of hydration the Polarizable Continuum Model (PCM) of Tomasi and co-workers [31] or the Langevin dipoles (LD) discrete approach of Warshel and Florián [32,33]. For both guanine and adenine, we envisaged the reaction at either the exocyclic amino group N^2 or N^6, respectively, or the N7 position, thus exhaustively performing 32 free energy calculations (Fig. 2).

Aralkylation of a purine base is not a trivial reaction and to determine 32 transition states would have been computationally too expensive. It is however to notice that it is nowadays possible to study chemical reactions in the condensed phase (crystalline or liquid) using hybrid potentials, allowing a part of the system to be treated at the molecular mechanics level and the other, where the precise knowledge of the electronic structure is crucial, quantum mechanically (for example, see Ref. [34] (enzymes, solutions), Refs. [35,36] (QM/MM reviews), Ref. [37] ($Cl^- + CH_3Cl$ S_N2 reaction in water)).

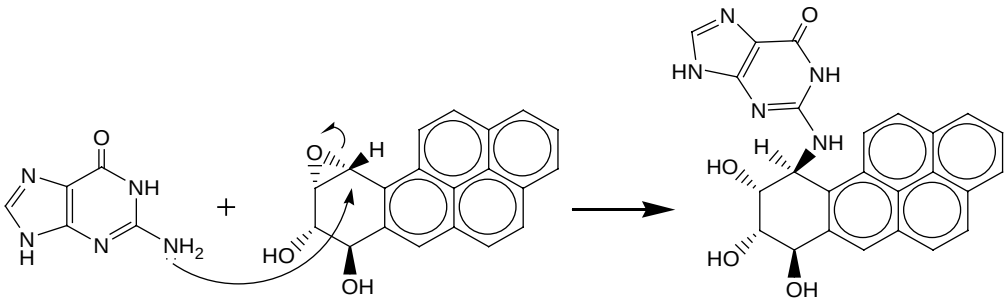

Fig. 1. Reaction between guanine and (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (S_N2 mechanism illustrated here).

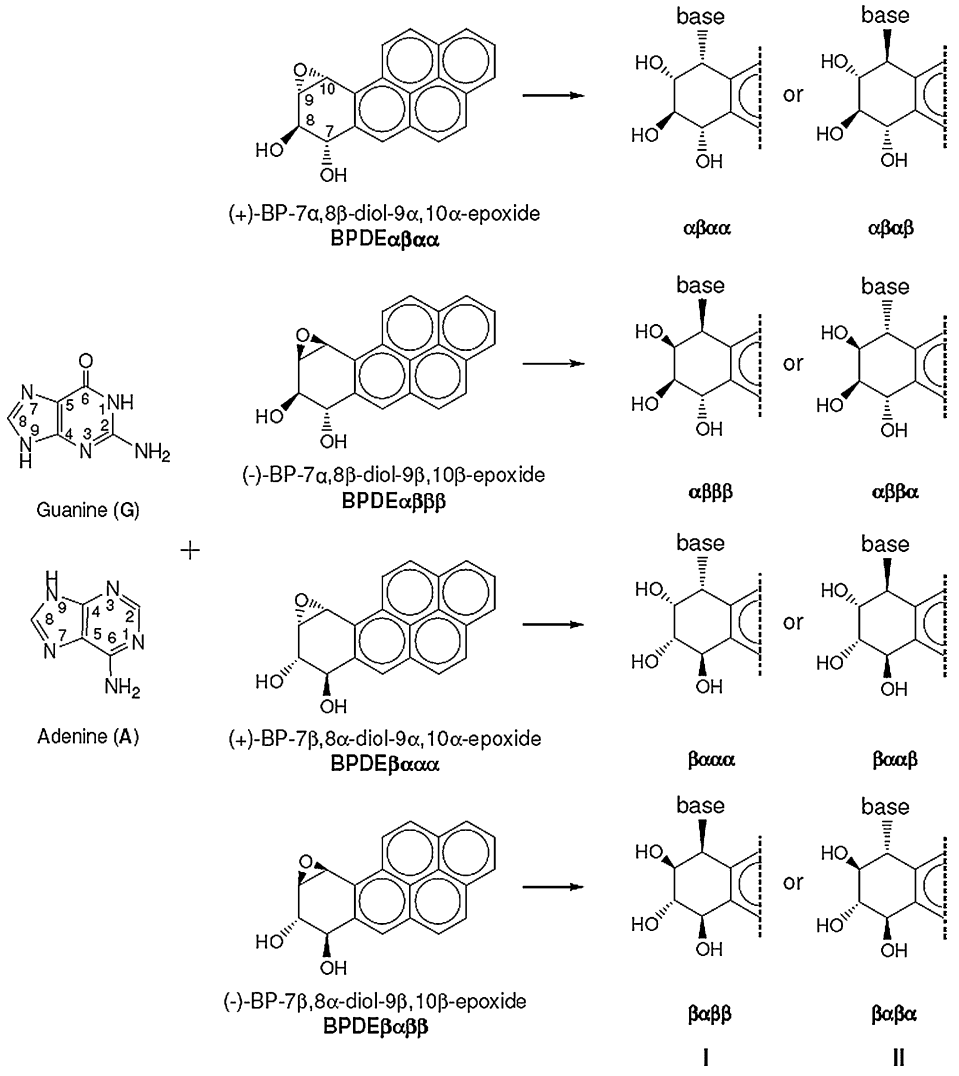

Fig. 2. Reaction between guanine (G) or adenine (A) and the four stereomers of benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE), derived from *trans*-benzo[*a*]pyrene-7,8-dihydrodiol. The bond between BPDE and the base is formed either on position N^2 or N7 of G or N^6 or N7 of A (N^2, N^6: superscript indicates that the nitrogen is linked to the carbon 2 or 6 of the base). α indicates a bond pointing below the average plane of the diol or triol cycle, β a bond pointing above. Stereotopology for each molecule is given accordingly, from positions 7 to 10 of the reactive cycle (bold). The reaction mechanism may be of either S_N1 or S_N2 type. For each given BPDE isomer, column I adducts (retention of position 10 configuration) can be formed only through a S_N1 mechanism, whereas adducts of column II through either S_N1 or S_N2 mechanisms (inversion of position 10 configuration).

QM/MM methodology would nevertheless have to be applied very carefully in this reaction series, because, as we will point out, the orientation of the base with regard to the triol moiety results in very specific interactions, the electronic behavior of the aromatic moiety has an incidence on the conformation of the adduct and on reactivity, and solvent molecules may hold a non-negligible role in the reaction process.

2. Computational methods

2.1. In vacuo calculations

All the calculations were performed using GAUSSIAN 03 suite of programs [38]. We performed in vacuo calculations on both the semiempirical MO level PM3 and Density Functional Theory (DFT) level B3LYP. Reactants were treated as separate species rather than complexes. Each reactant and product structure was first optimized at the PM3 level. For each adduct, between 2 and 6 starting structures were tested, i.e. with different initial orientations of the bases or of the hydrogens of the hydroxyl groups, given the fact that the resulting optimized structures for a given adduct set ended up with differences in energy ranging between 1 and 12 kcal mol^{-1}. In some cases this could be due to hydrogen bondings established differently. Such differences put under question mark the efficiency of PM3 method to describe accurately chemical processes involving labile systems which may converge into several secondary minima.

The structure with the minimum of energy was then systematically chosen as the input for B3LYP further optimization of the geometry, using 6-31G(d) basis set. This applied DFT level of theory is particularly suited for studying large systems of biological interest, as it is flexible enough with polarization functions applied to heavy atoms and

computationally not as demanding as MP2 or MP4 calculations. Becke3LYP is based on Becke's exchange functional [39] and Lee, Yang and Parr's correlation functional [40], thus allowing significant part of the electronic correlation energy to be taken into account. Vibrational analysis was performed in the harmonic approximation.

2.2. Hydration free energies

Two methods were applied to calculate free energies of hydration (ΔG_{hydr}) for the compounds we investigated. The solvent self-consistent reaction field of Tomasi and co-workers was based on the Polarizable Continuum Model, in which the solute is placed in a cavity formed by interlocking spheres centered on atomic groups [31,41–43]. Pauling radii were used and each sphere mapped with a number of tesserae defined to 100.

Langevin dipoles (LD) calculations were performed using ChemSol version 2.1 developed by Florián and Warshel [44], with proper parametrization. Unlike the PCM method, water molecules are defined by a grid of discrete polarizable LD embedding the solute. Merz–Kollman charges were calculated in PCM runs at B3LYP/6-31G(d) level, with the constraint to reproduce the dipole moment when fitting charges to the electrostatic potential (ESP). This set of point charges was then used to describe the solute, which was displaced 50 times in our calculations in the LD lattice, thus performing thermal averaging. The LD model uses van der Waals radii calibrated using observed solvation energies to determine free energy of hydration [32,33]. Florián et al. used LD for instance to study stacking and hydrogen bonding of nucleic acid bases in aqueous solution [45].

Solution reaction free energies (ΔG_{reac}) were the sum of B3LYP determined gas phase reaction energies (ΔE_{reac}), ΔZPE (zero-point energy) corrections and differences in free energies of hydration ($\Delta\Delta G_{hydr}$) obtained either with PCM or LD models. All our calculations were performed on either a multiprocessor Compaq ALPHA machine, or on Pentium IV 3 or 3.2 GHz PC's with 1.5 GB RAM.

2.3. Linear free energy relationships

Calculation of transition states and activation free energies [46,47] for 32 reactions would have been impracticable. Moreover, the BPDE/guanine or adenine reaction mechanism is quite complicated, involving several water molecules. Application of LFER to compare activation free energies within each set of guanine and adenine reactions looks ideal in the particular case of our study. Indeed the same nucleophilic substitution mechanism (although S_N1 or S_N2) is involved for all reactions together with completely structurally equivalent reactants, on the point of view of their molecular formula, being stereoisomeric forms of BPDE. The prerequisite criterion for this empirical approach is thus realized. LFER states that the reaction with the most favorable reaction free energy will have the lowest free energy of activation. This means that the more exothermic the reaction is, the faster. We detailed this point in a previous work [48]. Applications of LFER are found in fields as diverse as physical organic chemistry [49,50], chiral recognition mechanisms [51,52], enzyme catalysis [34] or in the study of different protein cooperative systems [53].

3. Results and discussion

Results of the calculated energy differences (kcal mol^{-1}) between reactants (guanine (G) or adenine (A) and the four stereomers of BPDE) and products (adducts at either *N*-cyclic or *N*-exocyclic positions of the bases) are presented in Table 1. In our notation, E refers to exocyclic, N to cyclic reaction on the amine of the base, and α and β bonds pointing below or above the plane of the molecule, respectively, the structures being viewed as in Fig. 2, i.e. epoxyde to the top and aromatic rings to the right, absolute configurations of the bonds with heavy atoms on carbons 7 to 10 of the triol cycle so given.

Let us comment first some trends in the free energy components of these reactions. Mean ΔZPE value was 2.64 ± 0.36 kcal mol^{-1}. As we may observe, there is a good agreement between PCM and LD obtained free energies of hydration differences for GE, GN and AE adducts, with an average absolute $\Delta\Delta G_{hydr}$ (PCM) minus $\Delta\Delta G_{hydr}$ (LD) value of 1.29 ± 0.64 kcal mol^{-1} over these three series. On the contrary, for the AN adduct series, strong differences appear in the $\Delta\Delta G_{hydr}$ values issued from both methods (see Appendix A).

The data of reaction energies at the different levels (PM3, DFT, in vacuum or solvent environment) reveal to be rather dispersed, and we shall thus highlight only the most obvious trends, by referring essentially to lowest and highest values in each series (indicated in bold normal and bold italics, respectively, in Table 1). ΔE_{reac} PM3 may only be compared to ΔE_{reac} B3LYP. While there is a good correspondence between PM3 and B3LYP for adenine adducts in the highest and lowest obtained values, it is not the case for guanine adducts, except for GEαβαα. B3LYP values are systematically higher, up to about 15 kcal mol^{-1} higher (AEαβαβ and ANαβαα).

The semiempirical PM3 method was applied because of low computational cost and to start with an already reasonably well optimized structure for performing DFT calculations. Moreover, this method is known for being in general in quite good agreement with DFT results for many systems of biological interest, and as we brought a particular attention in choosing in each case the structure over the sampled ones which presented the minimum of energy, we may try to see if some correlations exist with experimental observations. GEβααβ has the lowest value in GE series, and comes from *trans* opening of BPDEβααα, through S_N1 or S_N2 reaction. GEαβαα has the highest value in this series, a *cis* opening of BPDEαβαα appearing thus less favorable. We recall in Fig. 3 which adducts are the most probable to be formed according to current literature. In GN series, GNβααα has a value comparable to GEβααβ's one. As for GN only minor adducts were observed, the GNβααα PM3 result is somewhat contradictory, although for all other adducts values are higher than

Table 1
Free energy and free energy components for reactions between each of the four stereoisomers of benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE) and guanine or adenine, at both *N*-cyclic and *N*-exocyclic positions of the bases

Adduct	ΔE_{reac} PM3[a]	ΔE_{reac} B3LYP[b]	ΔZPE[c]	$\Delta\Delta G_{hydr}$ PCM[d]	$\Delta\Delta G_{hydr}$ LD[e]	ΔG_{reac} B3LYP PCM[f]	ΔG_{reac} B3LYP LD[g]
GEαβαβ	−31.73	−25.73	2.72	6.18	4.75	**−16.83**	**−18.26**
GEαβββ	−33.18	−22.29	1.92	5.61	4.46	−14.76	−15.91
GEβααα	−34.22	**−31.38**	3.03	11.72	13.52	−16.64	−14.84
GEβαβα	−33.40	−23.80	2.25	5.55	7.73	−15.99	−13.81
GEαβαα	***−31.45***	***−22.03***	2.64	6.53	8.59	−12.85	***−10.79***
GEαββα	−33.39	−23.57	2.76	8.72	6.51	***−12.09***	−14.30
GEβααβ	**−37.34**	−24.35	2.19	7.19	8.20	−14.97	−13.96
GEβαββ	−34.08	−26.74	2.53	8.63	9.15	−15.59	−15.07
GNαβαβ	−28.74	−25.92	3.02	11.11	12.48	−11.78	−10.41
GNαβββ	−30.53	***−17.58***	2.33	7.51	7.37	***−7.74***	***−7.88***
GNβααα	**−35.79**	−28.93	2.43	11.24	12.88	−15.25	−13.61
GNβαβα	−31.68	−23.76	2.22	7.92	9.14	−13.62	−12.40
GNαβαα	***−26.63***	−21.96	2.40	5.90	4.72	−13.66	−14.84
GNαββα	−30.99	−21.83	1.97	8.84	9.76	−11.02	−10.10
GNβααβ	−33.45	**−30.07**	2.36	11.79	12.61	−15.92	**−15.10**
GNβαββ	−32.67	−30.01	2.79	10.98	13.43	**−16.24**	−13.79
AEαβαβ	−35.40	−20.59	2.66	6.72	6.40	−11.21	−11.53
AEαβββ	−33.58	−22.25	2.29	4.94	3.16	−15.01	−16.79
AEβααα	**−37.55**	**−32.51**	3.43	11.01	12.20	−18.07	−16.88
AEβαβα	−33.79	−24.32	2.75	5.56	7.38	−16.01	−14.19
AEαβαα	***−27.45***	***−19.48***	3.21	6.16	5.16	***−10.11***	***−11.11***
AEαββα	−33.89	−20.13	2.40	2.92	1.98	−14.81	−15.75
AEβααβ	−36.29	−30.85	2.86	8.15	7.97	**−19.84**	**−20.02**
AEβαββ	−34.66	−25.36	2.78	5.86	7.49	−16.72	−15.09
ANαβαβ	−27.50	−20.43	2.68	3.84	−0.97	−13.91	−18.72
ANαβββ	−26.63	−14.92	2.84	3.26	−1.73	−8.82	−13.81
ANβααα	−30.65	−22.84	3.19	6.82	3.09	−12.83	−16.56
ANβαβα	−27.63	−20.43	2.68	3.85	−0.73	−13.90	−18.48
ANαβαα	***−24.16***	***−9.71***	2.69	1.37	−4.01	***−5.65***	***−11.03***
ANαββα	−24.88	−16.53	2.68	1.27	−6.64	−12.58	**−20.49**
ANβααβ	**−31.26**	**−26.52**	3.21	7.98	5.86	**−15.34**	−17.46
ANβαββ	−26.74	−20.41	2.72	3.37	−1.80	−14.33	−19.50

All energies are given in kilocalorie per mole, and were calculated as energy of the adduct minus the sum of the energies of the reactants (stereoisomer + base). G, A, E and N stand for guanine, adenine, *N*-exocyclic (reaction on position N^2 of guanine, N^6 of adenine) and *N*-cyclic (reaction on position N7 of guanine or adenine), respectively. α and β refer to the direction of hydroxyl bonds and bond with the corresponding nitrogen of the base, from positions 7 to 10 of the potential carcinogen (see Fig. 2).

[a] Gas-phase reaction energies (ΔE_{reac}) calculated at the semiempirical PM3 level.

[b] B3LYP/6-31G(d) calculated gas-phase energies.

[c] B3LYP/6-31G(d) ZPE corrections to the optimized structures obtained in b.

[d] Hydration free energy differences ($\Delta\Delta G_{hydr}$) determined using PCM SCRF at B3LYP/6-31G(d) level.

[e] Hydration free energy differences obtained using Langevin dipoles (LD) method with ChemSol 2.1 parametrization, Merz–Kollman charges being determined in PCM calculations.

[f,g] Reaction free energies: $\Delta G_{reac} = \Delta E_{reac}$ B3LYP + ΔZPE + $\Delta\Delta G_{hydr}$, the hydration term being issued either from PCM or from LD model. In bold normal: lowest values, in bold italics: highest values of a series.

the corresponding GE ones. AE adducts are also likely to be found, and when compared to the AN series, whose adducts were never reported, there would indeed be a preference for AE formation. Once more, BPDEβααα is involved, although in this situation a *cis* opening of the oxirane would occur, i.e. a S_N1 reaction, to give AEβααα. Strangely in all G and A series, adducts issued from a reaction with BPDEβααα stand apart from the others. We may conclude that, even though PM3 results are in gas phase and have nothing to do with free energy of a reaction, we can find here connections with experimental observations.

The rationale of LFER imposes the constraint that G and A series are not strictly comparable, but really allows to deduce relative free energies of activation from free energies of

Major adducts: **GE > AE**
Minor adducts: **GN**
Never reported: **AN**

(±) *anti*: *trans* opening (S_N1 or S_N2)
BPDEβααα → GEβααβ
BPDEαβββ → **GEαββα**

(±) *syn*: *cis* opening (S_N1)
BPDEαβαα → **GEαβαα**
BPDEβαββ → **GEβαββ**

Fig. 3. Major adducts formed with **BPDE** according to literature (see Section 1). **BPDE**βααα is the most mutagenic and carcinogenic reactant in general.

reaction, with proper electronic and hydration contributions taken into account. Reaction free energies, calculated as $\Delta G_{reac} = \Delta E_{reac}$ B3LYP + ΔZPE + $\Delta\Delta G_{hydr}$ (PCM or LD), show values (Table 1, f and g columns) about half the ΔE_{reac} PM3 ones. Except for GEαβαα (LD), AEαβαα and ANαβαα (PCM and LD), and ANβααβ (PCM) values, there is no match with highest or lowest PM3 values of a series. For GN adducts, the GNβααβ isomer appears with the most negative value, i.e. the lowest activation energy barrier (AEB), when computed with LD solvation model. Thus with guanine at N7 position, a *trans* opening of BPDEβααα is predicted by DFT (LD) to be a likely reaction to occur. This is strengthened by PCM approach with an even lower value, but GNβαββ (*cis* opening of BPDEβαββ) has the lowest AEB. GNαβββ is improbable to be formed, what is displayed quite clearly by both solvation methods. GN results are in conclusion consistent with literature data. For GE reactions, both PCM and LD ΔG_{reac} B3LYP values indicate that GEαβαβ has the lowest AEB. This value is even lower than those of GNβαββ or GNβααβ, what would be in agreement with a reaction with the exocyclic amine of guanine rather than N7. However *trans* opening of a *syn* stereoisomer has not been shown to be favorable (Fig. 3). GEαβαα and GEαββα are predicted to be the less favorable by the calculations, what does not correspond to observations. The adenine series of results reveal for AE adducts perfect concordance between lowest and highest PCM and LD values, with marked negative values for AEβααβ. This would strongly suggest that the adenine N^6 attack of BPDEβααα with *trans* opening of the epoxide ring is an easy reaction to take place, rather than *cis* opening of BPDEαβαα (high AEαβαα values). In the AN series, the strong variations between PCM and LD $\Delta\Delta G_{hydr}$ are of course reflected in PCM and LD ΔG_{reac} ones. Again, for PCM ΔG_{reac}, ANβααβ emerges, whereas ANαββα does in the LD series, the latter value being comparable to AEβααβ. Both adducts are the result of *trans* opening of their corresponding ± *anti* isomeric reactants, what is the mechanism encountered for G N^2 nucleophilic attack [5,9]. It nevertheless appears that products at N7 of adenine were up to our knowledge never seen yet. It might be that the especially pronounced atypical $\Delta\Delta G_{hydr}$ components of reaction free energy in LD approach is not a coincidence. Indeed charge effects, materialized in the dipole moment behavior, may affect hydration and subsequent reactivity properties. Striking is that $\Delta\Delta G_{hydr}$ (LD) of ANβααα and ANβααβ are the only positive values of this set of data (what here would have the effect of increasing AEB for the reaction with BPDEβααα), denoting important solvent effects. Curiously, the lowest values of $\Delta\Delta G_{hydr}$ are associated with among the highest ΔE_{reac} B3LYP ones, which tendency appears particularly in this series for the negative values, but in general also in the other series. This would tend to mean that the less favorable the reaction is in gas phase, the more favorable hydration will be. This nonetheless has not always the consequence of lowering ΔG_{reac} B3LYP value enough to lead to a favorable AEB.

It is interesting to examine the possible origins for calculated or observed reactivity levels. We give in 'Supplementary Material' an illustration of the incidence of specific structural molecular features on DFT ΔE_{reac} differences. An aspect which cannot be revealed just by comparing ΔE_{reac} PM3 or ΔE_{reac} B3LYP values is highlighted in Fig. 4. The four BPDE structures shown here are the result of B3LYP optimization. We see that epoxide rings are all in (pseudo)axial conformation, allowing efficient substitution reaction to occur [54]. But whereas for BPDEαβαα, BPDEαβββ and BPDEβαββ (Fig. 4a, b and d, respectively) hydroxyl groups are all (pseudo)equatorial, in BPDEβααα the hydroxyl on C7 is *anti* with respect to the oxirane (Fig. 4c). An anchimeric assistance mechanism might thus be envisaged only for this specific stereoisomer, which would favor nucleophilic attack by the base nitrogen. This facilitation mechanism has been nonetheless stated by Jerina et al. and Hulbert to occur on BPDEβαββ, and not BPDEβααα [55–57]. Indeed, in dry *tert*-butyl alcohol/DMSO, measurement of reaction rates with sodium *p*-nitrothiophenolate showed a more than 100-fold enhanced reactivity for BPDEβαββ compared to BPDEβααα. This was consistent with the NMR structure determination of BPDEβααα (THF used as solvent for the oxide synthesis [58]), which showed that the hydroxyl groups occupy mainly pseudo-equatorial positions, contrary to our own picture. From there it was deduced that the stereochemistry of BPDEβαββ was thus unequivocally established, and that it was similar to triptolide, an antileukemic diterpenoid triepoxide for which anchimeric assistance by a 14β-hydroxyl on the opening of the epoxide at 2 positions of distance seems to occur in a *syn* configuration. Anchimeric assistance is usually described as a *trans* process, but in this specific case, we hypothesize that the solvent probably plays a key role in mediating the opening of the oxirane by the 7-OH group of BPDEβαββ. Our optimization results, obtained in a water environment, rather are in favor of a *trans* process implying BPDEβααα, but this would deserve further investigation, using different dielectric constants (PCM) or Langevin dipoles, or by displaying a few discrete molecules nearby 7-OH and C10 and applying the QM/MM methodology for instance. This would help to precise the role of the solvent in the conformation of the dihydrodiol epoxide cycle and/or a possible mediation for the quite distant 7-OH in the anchimeric assistance on C10. A participation through an electronic effect of the adjacent aromatic cycle may not be excluded.

Reaction on C10 position of the diol moiety rather than C9 can be explained by (i) better stabilization of an intermediate C10 benzylic carbocation, (ii) the fact that OH(C8) is not *anti* with regard to the epoxide for BPDEαβαα and BPDEβαββ, and more specifically for BPDEβααα: (iii) possible hydrogen bond formation between H(O(C8)) and the epoxide oxygen (the distance would be 2.1 Å) favoring opening from C10 side, and (iv) greater steric hindrance with an attack on C9.

An important observation can be made when carefully looking at Table 2 ΔG_{hydr} PCM and LD values. Indeed in the four BPDE isomers, BPDEαβαα and BPDEβαββ are enantiomers, as well as BPDEαβββ and BPDEβααα. But whereas for the first pair PCM and LD ΔG_{hydr}'s are identical, they are different for the second, with a pronounced difference

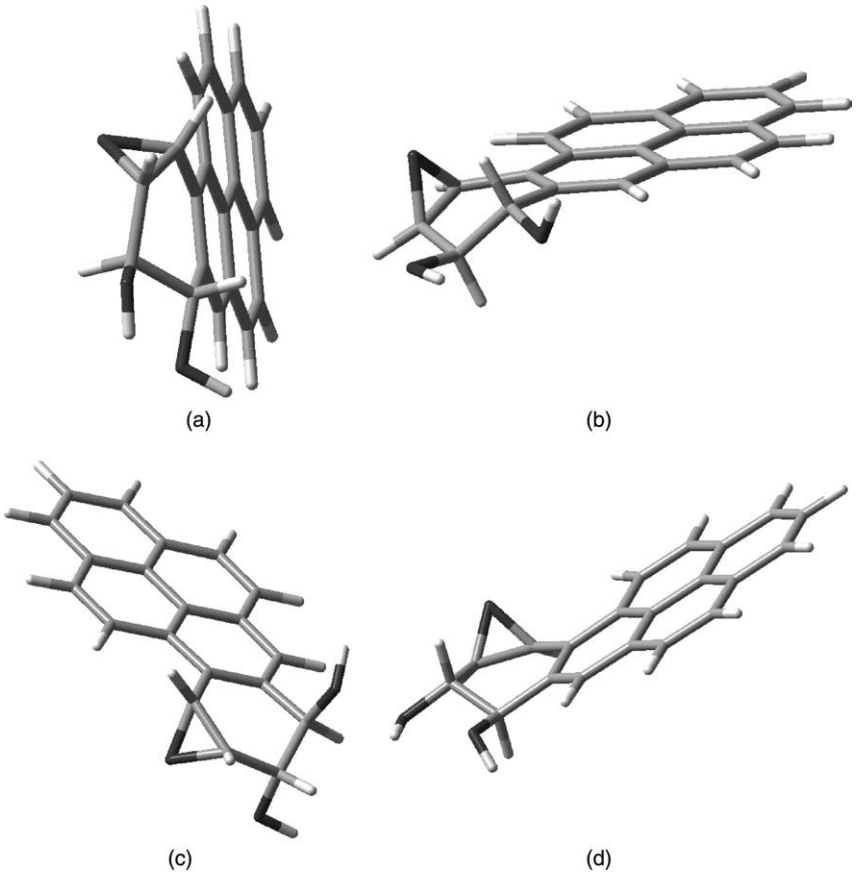

Fig. 4. B3LYP optimized isomers of BPDE. (a) BPDEαβαα; (b) BPDEαβββ; (c) BPDEβααα: [7-OH oxygen-C9] distance = 2.9 Å, [7-OH oxygen-C10] distance = 3.3 Å; (d) BPDEβαββ. Clearly, only for BPDEβααα may anchimeric assistance of nucleophilic attack on the oxirane by the 7-OH be possible, given that the two groups are *anti* and axial. Tube models obtained with Gaussview, with oxygens in black, carbons in dark grey and hydrogens in light grey.

in LD values. However, enantiomers should have equal properties in non-chiral environment: solvation free energies should be equal. The answer for this calculated difference is revealed in Fig. 4, where the shown B3LYP optimized structures were used for ΔG_{hydr} evaluation. Indeed where hydroxyls are (pseudo)equatorial, hydration energies are comparable (slightly higher for BPDEαβββ), whereas for BPDEβααα, in which the 7-OH is axial, hydration energy is markedly lower.

These differences can also be correlated to the energies of the optimized isomers. Indeed we obtained a PM3 minimized energy for BPDEβααα of ~ -0.0346 Hartree, whereas for the other three isomers an identical value of ~ -0.0412 Hartree, what is an about 4.1 kcal mol^{-1} difference. BPDEβααα thus lowers the ΔE_{reac} values of βααα or ββαα adducts, what is verified in all the series (see Table 1). The fact that the PM3 determined energy of BPDEβααα is significantly higher than the one of the other stereoisomers (what is actually confirmed by DFT optimization results, where the corresponding difference amounts to $\sim$6.6 kcal mol^{-1}) indicates that this molecule is less stable, i.e. might be more reactive with regard to G or A. This is consistent with the particular observed mutagenicity and carcinogenicity of this compound, and might explain its greater 'reactivity' also with regard to the model water environment. The fact that as well PM3 as DFT optimizations give analogous energy differences between BPDEβααα and the other isomers suggests that the two computational methodologies we have used in this work do not lead to an error intrinsic to one or the other. Of course, other computational studies (vide supra) will have to be performed, and new experimental investigations may help to further precise the stereochemical characteristics of these isomers, in relation to their physicochemical properties, with particular focus on their specific solvent or molecular interactions. The question is of great importance for complex stereoisomeric molecular systems, as in our case where a cycle is constrained by a rigid aromatic moiety.

Table 2
Free energies of hydration (ΔG_{hydr}) determined for BPDE isomers with either PCM or LD methods. Energies are given in kcal/mole

Reactant	ΔG_{hydr} PCM	ΔG_{hydr} LD
BPDEαβαα	−19.36	−16.54
BPDEαβββ	−19.04	−14.97
BPDEβααα	−22.56	−20.00
BPDEβαββ	−19.36	−16.63

4. Conclusions

Up to now, only a few attempts were aimed at building up an overall picture of carcinogenic DNA adduct formation and developing a conceptual framework based on common structure/reactivity features [14,59]. In our present work, we computed at a medium high DFT level of theory reaction free energies of formation of guanine and adenine adducts with the four in vivo encountered stereoisomers of benzo[*a*]pyrene-7,8-diol-9,10-epoxide ultimate carcinogen in a systematic study of 32 possible purine bases products at their cyclic or exocyclic amine positions. Linear free energy relationships allowed us to compare the activation free energy barriers in each guanine or adenine set of results, due to structural closeness and identical reaction mechanism. Differences between lowest and highest values in each series were important enough to be able to outline major trends and confront them to observations. Many factors have to be kept in mind when analyzing experimental data, such as concentration of the species, much more complex environments, reactivity toward the whole DNA, local acidobasicity conditions…, which play an important role as for the variability and significance of BPDE/DNA reactions the way they are apprehended. Hence, it is difficult to be convinced that what applies to a given case may apply to another, and that the ensemble of data in the literature are strictly generalizable. Conscious of these limitations, and basing ourselves on the strongest trends in our data, we found (i) for N^2-guanine reaction, the most likely to occur, that GEαβαβ has the lowest activation energy barrier, lower than the lowest guanine N7 adduct one, which is consistent with experiments but does not correspond to the most frequently encountered mechanism; (ii) for N7-guanine reaction mentioned as minor, as well as (iii) for N^6-adenine quite probable reaction, very correct correlation with experimental results, BPDEβααα opened *trans* being the most favorable situation; finally (iv) for the never reported N7-adenine reaction, solvent effects clearly evidenced by LD method (but not by PCM method) that may prevent reactions elsewhere the most likely. We moreover demonstrated that solvation free energies were tightly linked to the molecules total dipole moments squared, with a linear relationship, allowing to better understand the AN LD $\Delta\Delta G_{hydr}$ results. Except for a limited number of values, the semiempirical PM3 method did not correlate well with B3LYP reaction free energies and only displayed good agreement within highest and lowest values with adenine B3LYP gas phase results. Nevertheless it showed not to be unconnected with experimental observations and BPDEβααα was in all the series leading to the lowest reaction energy values. Finally our hypothesis of *trans* anchimeric assistance by the benzylic 7-hydroxy group, that would enhance the reactivity of BPDEβααα, could be one plausible explanation accounting in an aqueous environment for the generally much higher in vivo mutagenicity and carcinogenicity observed for this particular isomer, but solvent effects will have to be precised.

Our present computational effort shows to be very promising for a better understanding of the reactivity of complex molecular systems in a systematic approach, revealing interesting properties to be further investigated. It might be easily transposed to other reactions involving structurally similar reactants and products with analogous reaction mechanisms. It is worth to emphasize that in this study we considered the ultimate carcinogen and relevant part of DNA. This allowed us to account for the stereochemical aspect showing up at this stage, whereas many carcinogenicity QSAR studies, based on the graph theory or a π electron method like Hückel molecular orbital theory, are in general focusing on procarcinogens [59]. Thus, the QSAR molecular descriptors which are used, although of course useful as they are adopted because they give good connection with experimental results, may not be fully justified [60].

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Appendix A

We tried to understand the reason for the observed discrepancies between $\Delta\Delta G_{hydr}$ values obtained with either PCM or LD methods for the AN adducts. The explanation came from the analysis of the total dipole moment (μ_{Tot}) behavior of the molecules, calculated during each PCM SCRF run. As an approximation, free energy of hydration may be expressed as $\Delta G_{hydr} \sim -k\mu_{Tot}^2/R^3$ according to Kirkwood–Onsager model (dipole in a spherical cavity immersed in a dielectric continuum), where R is the radius of the cavity and k a constant. Values of free energies of hydration (ΔG_{hydr}) computed with PCM or LD methods for the two G and two A series are plotted in Fig. A1. We first remark that there is a linear dependence between μ_{Tot}^2 and, ΔG_{hydr} with in the case of adenine two distinct slopes relating to each hydration free energy calculation model. Still in this latter case, we secondly see that the data define two regions. The first corresponds to values of μ_{Tot}^2 ranging from 0 to $\sim$100 D^2, which belong to the AE series, where the differences between ΔG_{hydr} (LD) and ΔG_{hydr} (PCM) are quite high, increasing to $\sim$10 kcal mol^{-1} when μ_{Tot}^2 tends to zero. The second concentrates between $\sim$100 and 200 D^2, which is the interval associated to the AN series, where ΔG_{hydr} differences are much smaller. (All the numerical values are given in Supplementary Material, Table 3). It turns out that the average AE ΔG_{hydr} difference between values of PCM and LD series compensates the sum of the differences observed for A and BPDE (average), explaining comparable AE $\Delta\Delta G_{hydr}$ PCM and LD values. The same applies to the G series: in μ_{Tot}^2 plots, points are much more dispersed, but the difference along the regression lines is about constant, and the same compensation occurs between reactants and adducts. This is not the case for the AN series, where the average PCM/LD $\Delta\Delta G_{hydr}$ difference is 4.84 kcal mol^{-1}, due to small ΔG_{hydr} differences. About the same two regions can be defined for the G series, but contrary to the A series, GN values concentrate in the 0$\sim$100 D^2 range, whereas GE values in the $\sim$100 to 200 D^2 interval (Fig. A1).

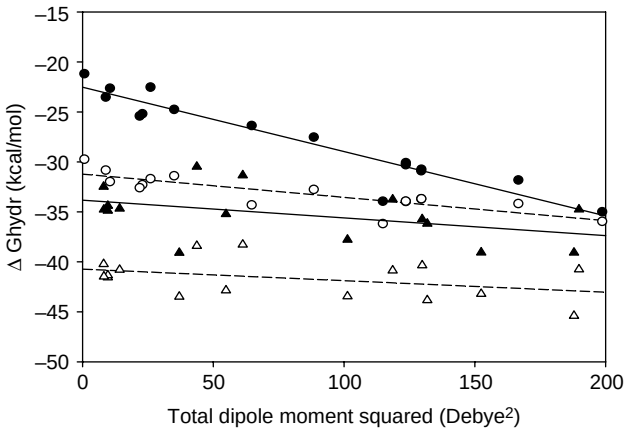

Fig. A1. ΔG_{hydr} calculated with PCM (○, △) or LD (●, ▲) methods for both *N*-cyclic and *N*-exocyclic BPDE/adenine adducts (circles) and *N*-cyclic and *N*-exocyclic BPDE/guanine adducts (triangles) as a function of their total dipole moment squared ($\mu_{Tot}{}^2$ values from Table 3 in 'Supplementary Material'). The corresponding linear regressions are shown (LD, full lines; PCM, dashed lines), with slope values, from top to bottom, of −0.064, −0.023, −0.018 and −0.012.

One contribution for the difference observed in the LD BPDE/A adducts reported slope of the linear regression through ΔG_{hydr} points might be related to the fact that in LD calculations Merz–Kollman charges are used as an input, and to the constraint already mentioned that these charges are forced to reproduce the dipole moment when fitted to the ESP. For the AN adducts, specific electronic effects due to particular orientations of the adenine moiety are certainly more properly taken into account by the LD method as for the interactions with the solvent (discrete dipoles). Indeed, it might be that for the negative $\Delta\Delta G_{hydr}$ values obtained, hydration is facilitated by a favorable orientation of the Langevin dipoles with regard to the molecules, overall or toward some specific regions. We may conclude on this particular aspect that this ΔG_{hydr} vs. $\mu_{Tot}{}^2$ linear dependence could be observed only because we are in presence of series of structurally identical molecules, whose total dipole moments were moreover well distributed over the values windows.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.theochem.2006.02.005

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