MOLECULAR PHARMACOLOGY, 22:459–464

Molecular Orbital Studies of Epoxide Stability of Carcinogenic Polycyclic Aromatic Hydrocarbon Diol Epoxides

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Received September 28, 1981; Accepted April 15, 1982

SUMMARY

Semiempirical molecular orbital calculations (by modified neglect of diatomic overlap method) were performed on diol epoxide metabolites of five polycyclic aromatic hydrocarbons (PAHs)—benzene, naphthalene, phenanthrene, chrysene, and benzo[*a*]pyrene (BP)—to gain insight into the various carcinogenic potencies of these compounds. Opening of the epoxide rings of the diol epoxides was calculated to be exothermic for all of the PAHs investigated. The bay-region diol epoxides of BP were calculated to open spontaneously to the triol carbonium ion upon protonation. For the bay-region *trans*- and *cis*-diequatorial diol epoxides of 5-methylchrysene the methyl group destabilized the epoxide. These results suggest that the conformation of the saturated, angular benzo-ring is important in determining bay-region epoxide stability. Conformational flexibility of the aromatic ring system is offered as one reason for partial stabilizing of bay-region epoxides. These results also suggest that the existence and potentiation of PAH carcinogenicity is correlated with the lack of stability of the bay-region epoxide ring. Considerations of thermochemical stability have value in predictions of carcinogenic potency.

INTRODUCTION

Many PAHs[1] become cytotoxic, mutagenic, and carcinogenic upon biotransformation to reactive intermediates (1, 2). Carcinogenic PAHs, which contain a bay-region angular benzo-ring dihydrodiol epoxide, with one carbon atom of the epoxide ring in the bay region, are the ultimate carcinogenic metabolites (3–6). Both the mutagenicity and carcinogenicity of PAHs have been correlated with ease of epoxide ring opening and consequent carbonium ion formation from the diol epoxide metabolities, calculated by the PMO method (7–9).

Epoxides adjacent to the bay region were calculated to be the most reactive of the several possible isomeric diol epoxides of a parent hydrocarbon. However, because the PMO method fails to incorporate hydroxy-group contributions, the PMO calculations do not differentiate between values of $\Delta\epsilon_{deloc}/\beta$, the π-electron energy change associated with epoxide ring opening to carbonium ion, for diol epoxides versus the structurally analogous tetrahydro epoxides. The PMO method is adequate for calculating properties of planar aromatic hydrocarbons but can give inaccurate results for nonplanar, saturated hydrocarbons with angular, puckered benzo-rings and for the various dihydrodiols, dihydrodiol epoxides, and carbonium ions of the PAHs (10). Furthermore, the PMO method accounts only for π-electrons.

[1] The abbreviations used are: PAHs, polycyclic aromatic hydrocarbons; PMO, perturbational molecular orbit; MNDO, modified neglect of diatomic overlap; BP, benzo[*a*]pyrene.

To provide insight into PAH carcinogenicity, we therefore sought an alternative method for calculating epoxide stability of PAH diol epoxides. *Ab initio* calculations of the energetics of epoxide ring opening for the oxiranium cation indicated, from the relative energy of the closed and opened epoxide ring forms and the size of the barrier to interconversion, that the acid-catalyzed reactivity of oxirane was A2 (bimolecular rate-limiting step) rather than A1 (unimolecular rate-limiting step) (11). This conclusion is consistent with experimental results which support an A2 acid-catalyzed mechanism for oxirane hydrolysis. Thus calculated energetics of epoxide ring opening can be used to predict a reaction mechanism. Because of the molecular size of the PAHs we intended to investigate, a semiempirical method less time-consuming than *ab initio* methods was required to investigate adequately the potential metabolites. *Ab initio* methods have been used to investigate bay-region PAH diol epoxides (12, 13), but a full examination of the potential energy surface has not been possible because of the need for arbitrary assumptions about the molecular geometry.

The molecular orbital MNDO method was chosen because it employs the closest approximation to the Hamiltonian of any zero-differential overlap method (i.e., neglect of diatomic differential overlap) and because it effectively predicts many experimental properties, especially heats of formation. MNDO adequately treats the correlation energy for the closed-shell ground-state wave function (14) and provides an accurate description of three-membered rings, including oxirane (15). Other sem-

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iempirical methods have been applied to the reactivity of bay-region diol epoxides (16, 17), but known deficiencies in these methods, particularly an inadequate treatment of epoxide stability with some of the methods (18), make the results equivocal.

We have investigated the epoxide ring opening of several representative PAH diol epoxides and the corresponding triol carbonium ions through the calculated energetics. Benzene was selected for determination of the behavior of a diol epoxide on a single aromatic ring. Naphthalene was included to determine the influence of an intact adjacent aromatic ring. Phenanthrene, chrysene, and BP were selected because all have bay regions but exhibit varying carcinogenic potencies (19). Phenanthrene, which is not carcinogenic, is the prototype bay-region PAH, and chrysene and BP differ by having one or two additional fused aromatic rings, respectively.

We have applied MNDO to these representative PAH metabolites to test the PMO-based suggestion of a correlation of ease of carbonium ion formation with carcinogenicity. MNDO accounts for σ- and π-type electrons, and the use of this more justifiable theoretical method should provide greater insight into epoxide stability and the role of the bay region in PAH carcinogenicity.

METHODS

The MNDO is parameterized to reproduce experimental heats of formation (18, 20). Each metabolite was geometry-optimized totally so that the results would be consistent for comparisons between metabolites. The mean absolute error in the computed MNDO heats of formation is 6.3 kcal/mole. In comparisons of the energies of similar compounds, the errors in the heats of formation generally cancel out. MNDO with geometry optimization has been demonstrated with some molecules to give more accurate energy differences than some *ab initio* treatments (18, 20). We employed the Quantum Chemistry Program Exchange version of MNDO (21). Computations were performed on a VAX 11/780 computer in the Division of Laboratories and Research, New York State Department of Health.

Calculations were made by using the ground-state singlet determinant. For the protonated diol epoxides a trial geometry, with the proton placed on the oxygen lone-pair axis at an oxygen-proton distance of 0.95 Å, was used. An initial geometry optimization was performed with an epoxide carbon-carbon-oxygen angle arbitrarily set at 60°. This angle was then optimized as a final step in the calculation. The conformation of the dihydrodiol groups was checked visually by use of the graphics program NAMOD (22) to ensure authentic diaxial or diequatorial hydroxy conformations.

RESULTS AND DISCUSSION

The angular, saturated benzo-ring of a PAH puckers in the half-chair conformation, and as a consequence there are four diol epoxide isomers, or eight if enantiomers are included (Fig. 1). Isomers designated as *cis* or *trans* have the benzylic hydroxy group and the epoxide oxygen atom on the same side (*cis*) or on the opposite side (*trans*) of the angular benzo-ring. The dihydrodiol group, if enzymatically formed by the successive action

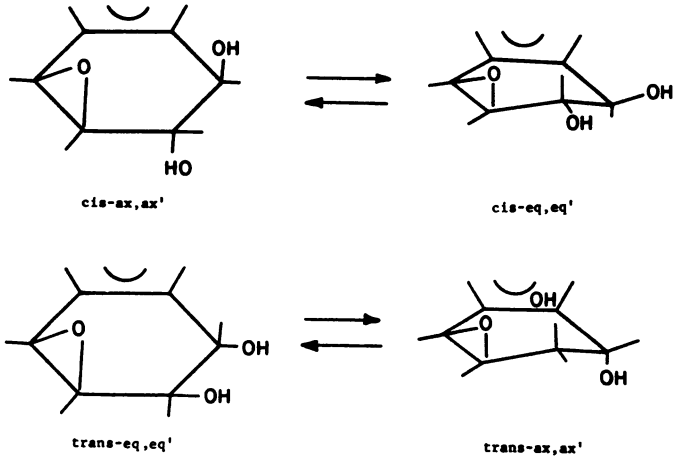

FIG. 1. *Diol epoxide isomers of a PAH angular benzo-ring*
Ax, Axial; *eq*, equatorial. *Cis* and *Trans* refer to the relationship between the benzylic hydroxy group and the epoxide oxygen atom.

of cytochrome P-450 and epoxide hydrolase, has the hydroxy groups *trans* (1).

Trans-dihydrodiols, in the absence of steric restrictions, prefer a diequatorial conformation of the hydroxy groups (23). Theoretical calculations with the model compound 3,4-epoxy-5,6-dihydroxycyclohexene, using the MINDO/3 method, indicate that the *trans*-diol epoxide prefers a diequatorial conformation of the hydroxy groups, whereas the *cis*-diol epoxide prefers a diaxial conformation (24). However, the *trans*-diol epoxide of this compound is less stable than the *cis*-diol epoxide.

Protonation facilitates epoxide ring opening to a carbonium ion, and it is possible to determine a reaction coordinate for triol carbonium ion formation from the protonated diol epoxide (Fig. 2). In this study we investigated the potential energy surface of the protonated diol epoxide in its immediate neighborhood and characterized it as either a local minimum with a calculated energy or a saddle point. Activation energies for epoxide ring opening were not systematically investigated.

We calculated the diol epoxide-carbonium ion equilibrium for *trans*-diol epoxides of benzene, naphthalene, phenanthrene, chrysene, and BP (Fig. 3), since the *trans*-isomers are the most potent carcinogens (5). The last three compounds have the epoxide ring in the bay region. The results are summarized in Table 1. The difference in heats of formation ΔE_f (for the reaction in Fig. 2) is defined as ΔH_f (carbonium ion) $-$ ΔH_f (protonated diol epoxide) and represents the energy change when carbonium ion is formed from the protonated epoxide ring of the PAH diol epoxide. Both conformations of the *trans*-

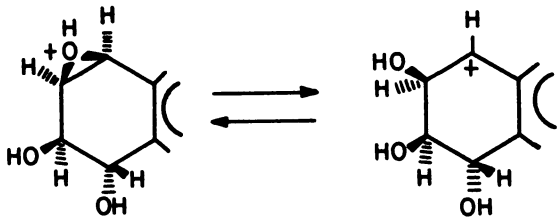

FIG. 2. *Ring opening of protonated diol epoxide to triol carbonium ion of a PAH*
A *trans*-diol epoxide is depicted.

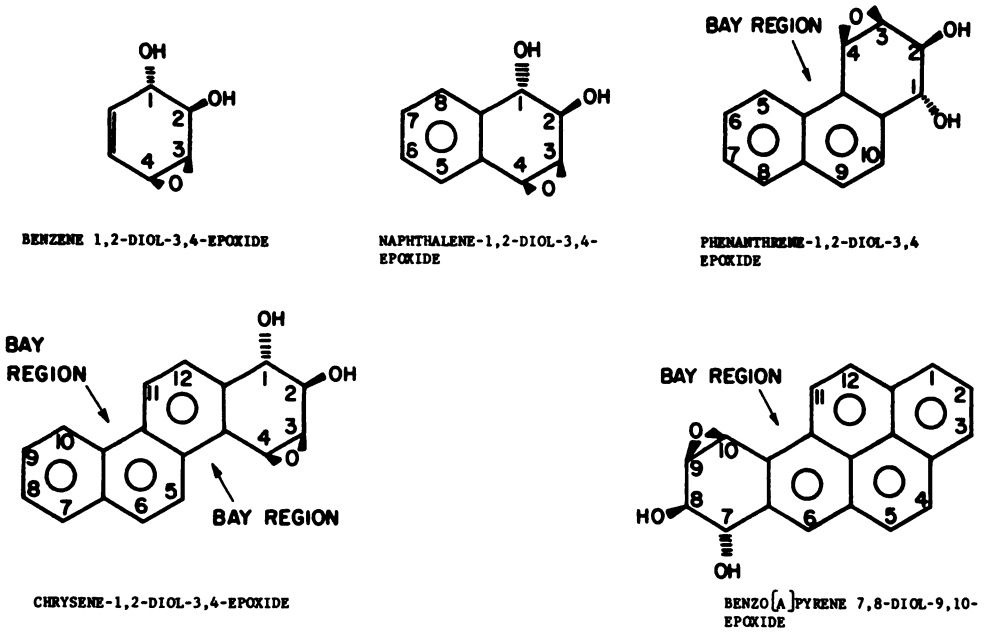

FIG. 3. *Diol epoxides of PAHs investigated in the study*
Nomenclature according to Dipple (19).

diol epoxide and triol carbonium ion were calculated. Ring opening was exothermic for each PAH examined.

The enthalpy change or reaction heat of carbonium ion formation can be compared with the previously reported trend in the resonance energy of delocalization, $\Delta\epsilon_{deloc}/\beta$, computed by the PMO method (7, 8). The formula $\Delta\epsilon_{deloc}/\beta$ represents the π-energy change in delocalization associated with ring opening and is strictly a reactant-product enthalpy difference. However, the Bell-Evans-Polanyi principle proposes a parallel reaction between activation energy and reaction heat. By this relationship a rate or "ease" of carbonium ion formation can be inferred from $\Delta\epsilon_{deloc}/\beta$.

The calculated triol carbonium ion structures are given in Figs. 4 and 5. All of the diol epoxide and triol carbonium ion structures are clearly defined with respect to

TABLE 1

Carbonium ion formation from PAH diol epoxides calculated from MNDO heats of formation (ΔH_f)

PAH	ΔH_f		ΔE	Approximate relative carcinogenicity[a]	$\Delta\epsilon_{deloc}/\beta$[b]
	Protonated diol epoxide	Triol carbonium ion			
	kcal/mole		*kcal/mole*		
Benzene				NA[c]	NA[c]
trans-eq,eq′	91.9	75.0	−16.9		
trans-ax,ax′	92.0	73.8	−18.2		
Naphthalene				−	0.488
trans-eq,eq′	100.9	78.0	−22.9		
trans-ax,ax′	99.0	75.6	−23.5		
Phenanthrene				−	0.658
trans-eq,eq′	120.4	90.8	−29.6		
trans-ax,ax′	117.6	90.1	−27.5		
Chrysene				+	0.640
trans-eq,eq′	136.8	107.3	−29.5		
trans-ax,ax′	134.1	105.0	−29.1		
cis-eq,eq′	137.0	107.9	−29.1		
cis-ax,ax′	136.9	108.2	−28.7		
Benzo[*a*]pyrene				++++	0.794
trans-eq,eq′	—[d]	104.8	—[d]		
trans-ax,ax′	—[d]	104.6	—[d]		
cis-eq,eq′	—[d]	104.7	—[d]		
cis-ax,ax′	—[d]	105.9	—[d]		

[a] Based on carcinogenicity indices given in Lehr and Jerina (9). −, inactive; +, weakly active; ++++, strongly active.
[b] From Lehr and Jerina (9).
[c] No information available; benzene is a suspected human leukemogen.
[d] Collapses spontaneously to triol carbonium ion.

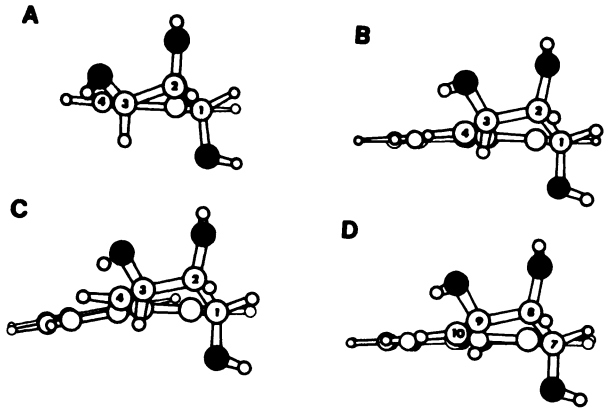

FIG. 4. *Trans-ax, ax' triol carbonium ions*
○, Carbon; ●, oxygen; ◦, Hydrogen. Carbon atoms are numbered as in Fig. 3. A, naphthalene; B, phenanthrene; C, chrysene; D, BP.

equatorial or axial placement about the saturated, angular benzo-ring and do not vary much from compound to compound.

In the three bay-region diol epoxide metabolites, as the epoxide ring becomes increasingly unstable to protonation, carcinogenicity increases: phenanthrene, noncarcinogenic (19); chrysene, weakly carcinogenic (6, 19); BP, strongly carcinogenic (5, 19). In phenanthrene the diaxial and diequatorial isomers of the bay-region protonated diol epoxide are thermochemically stable. Similarly, both *trans*-isomers of the chrysene bay-region diol epoxide are stable. For BP, both bay-region protonated diol epoxides collapse without activation energy to the ring-opened triol carbonium ion. Stability is also influenced by other factors discussed below.

A possible explanation for the enhanced stability of the protonated diol epoxide of chrysene over BP is that the bay-region hydrogen 5 adjacent to the bay-region epoxide hydrogen can move to accommodate a sterically strained situation (a nonbonded hydrogen-hydrogen distance less than twice the van der Waals radius). This is made possible by conformational flexibility of chrysene's aromatic rings (Fig. 4, see twisted rings). Such flexibility is absent in the aromatic pyrene ring system of BP because of the extra aromatic ring. Similarly, the aromatic-ring flexibility of phenanthrene can account for the stability of the *trans*-diaxial and *trans*-diequatorial bay-region diol epoxides. Figure 6 illustrates the two phenanthrene *trans*-diol epoxides resulting from this conformational flexibility. Thus the changing ring flexibility and its effect on epoxide stability may explain the increase in carcinogenic potency from phenanthrene to chrysene to BP.

Whalen *et al.* (25) have suggested that steric repulsion of the bay-region hydrogen atoms (e.g., H-4,5 for chrysene; H-10,11 for BP) increases in the *trans*-diequatorial relative to the *trans*-diaxial diol epoxide conformation. The consequences of this relative stability are illustrated by phenanthrene and by chrysene (Table 1).

Cis-BP 7,8-diol-9,10-epoxide is expected to be unstable in both of its isomeric forms (Fig. 1) because of the conformational inflexibility of the pyrene aromatic nucleus. Calculations for both *cis*-isomers confirm this expected ring opening of the bay-region epoxide. Like the *trans*-BP protonated diol epoxide, the *cis*-BP diol epoxide collapses spontaneously to the triol carbonium ion with no activation energy. The calculated heats of formation of these ions are given in Table 1. Results for the *trans*-chrysene diol epoxide suggested that *cis*-chrysene 1,2-diol-3,4-epoxide would be stable in both conformations. Calculations confirm this prediction (Fig. 1; Table 1).

The bay-region *trans*-diol epoxides of chrysene 1,2-diol-3,4-epoxide would be expected to be destabilized by substitution with a methyl group at carbon atom 5, since this electron-donating group should affect the activation energy of only 2 kcal/mole, which was found for ring opening of the *trans*-diequatorial isomer. A similar destabilization effect is likely for the *trans*-diaxial isomer. For the *trans*-diaxial 5-methyl isomer (with two possible conformational situations of the 5-methyl group relative to hydrogen atom 4), both conformations (Fig. 7) were calculated to have stable protonated diol epoxides. The coplanar conformation, with a heat of formation of 138.2 kcal/mole, is 0.2 kcal/mole more stable than the twisted conformation. However, the *trans*-diequatorial isomer was unstable, and collapsed to the bay-region triol carbonium ion. The *cis*-diequatorial isomer was also destabilized by the presence of the methyl group at carbon

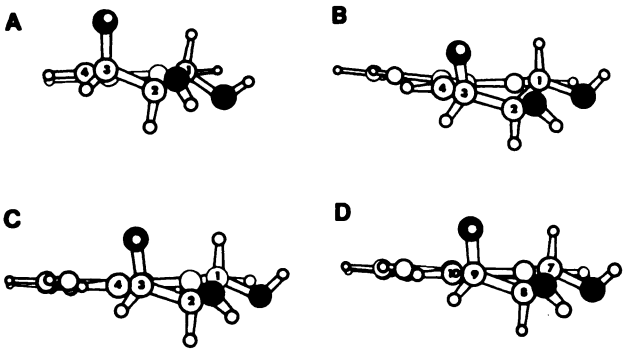

FIG. 5. *Trans-eq,eq' triol carbonium ions*
○, Carbon; ●, oxygen; ◦, hydrogen. Carbon atoms are numbered as in Fig. 3. A, Naphthalene; B, phenanthrene; C, chrysene; D, BP.

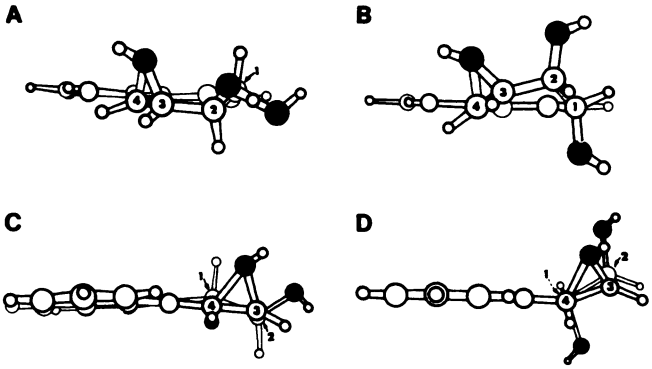

FIG. 6. *Trans-protonated bay-region diol epoxides of phenanthrene*
○, Carbon; ●, oxygen; ◦, hydrogen. Carbon atoms are numbered as in Fig. 3. A and C, Alternate views of *trans*-eq,eq' phenanthrene diol epoxide; B and D, alternate views of *trans*-ax,ax' phenanthrene diol epoxide.

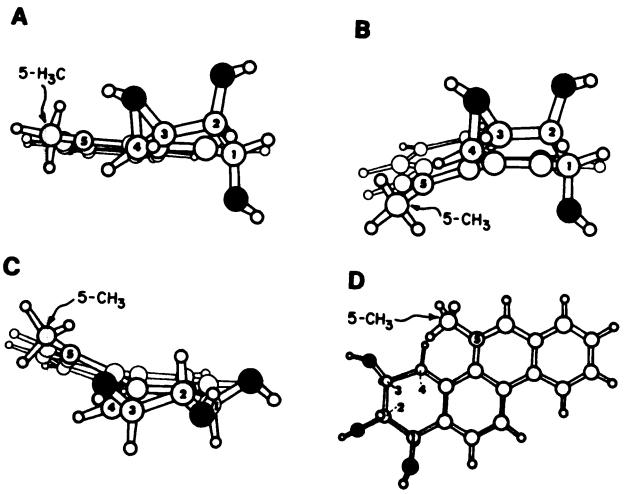

FIG. 7. *5-Methylchrysene protonated bay-region diol epoxide and triol carbonium ion*

A, Coplanar conformation 5-methyl-*trans*-ax,ax′ diol epoxide; B, twisted conformation 5-methyl-*trans*-ax,ax′ diol epoxide; C, 5-methyl *cis*-eq,eq′ triol carbonium ion; D, 5-methyl *cis*-eq,eq′ triol carbonium ion.

atom 5 (the structure of the 1,2,3-triol carbonium ion is given in Fig. 7). 5-Methyl chrysene is a potent carcinogen (6), approximately equivalent to BP. The calculated methyl-induced instability of the chrysene bay-region diol epoxides suggests that 5-methylation potentiates the carcinogenicity of chrysene by destabilizing its bay-region diol epoxide.

Although the bay-region diol epoxide of BP is unstable and collapses spontaneously to the triol carbonium ion, it is possible to calculate the energy loss from this ring opening from the energy of the diol epoxide at a carbon-carbon-oxygen angle of 60°. This energy loss is 36 kcal/mole, consistent with extrapolation from the other PAHs (see Table 1).

The data in Table 1 indicate that carcinogenicity can be correlated with the susceptibility of the bay-region epoxide ring of a PAH diol epoxide to open with an S_N1 mechanism upon protonation. The role of a carbonium ion as a carcinogenic agent has been suggested (26). However, our calculations—which indicate that both *cis*- and *trans*-diol epoxides of BP are unstable, yielding carbonium ions—suggest that the instability alone does not explain the differing carcinogenic potencies of the *cis*- and *trans*-isomers. An alternate explanation is that there is more extensive biotransformation in the *trans*- than in the *cis*-diol epoxide, resulting from the stereospecificity of cytochrome P-450 and epoxide hydrolase (27). As yet unexplained is why the administration of *trans*-BP diol epoxide to newborn mice causes high tumorigenicity, whereas an equimolar amount of *cis*-BP diol epoxide causes negligible tumorigenicity (5).

The lack of carcinogenicity of phenanthrene, which is the prototype bay-region PAH, can be attributed to its stable protonated epoxide ring in the bay region. The diol epoxide of phenanthrene thus could react in an S_N2 manner with a noncritical cellular nucleophile. Phenanthrene and BP differ structurally in that the latter has two additional fused aromatic rings, which are sufficient to abolish the activation energy for bay-region epoxide ring opening and stabilize the bay-region triol carbonium ion. The triol carbonium ions of phenanthrene and BP have virtually identical net charges on the benzylic carbon.

Several lines of experimental evidence are in agreement with the conclusion that carcinogenic bay-region PAH diol epoxides are unstable to protonation. Naphthalene *trans*-diol epoxide is readily synthesized, but preparation of BP diol epoxide is made difficult by its high reactivity (23). Our results suggest that the diol epoxide-carbonium ion equilibrium is shifted to the diol epoxide in the case of naphthalene and to the triol carbonium ion in the case of BP. BP diol epoxide rapidly hydrolyzes to tetraol in aqueous solution (28), whereas the diol epoxides of naphthalene are relatively stable in water (2).

Experimental results on the kinetics of hydrolysis of BP diol epoxide to tetraols led to the conclusion that *trans*-BP diol epoxide can undergo an S_N1 reaction mechanism (26). A carbonium ion was suggested as an intermediate in an acid-catalyzed first-order reaction. Further kinetic studies of the hydrolysis of BP diol epoxide in phosphate suggested that $H_2PO_4^-$ acts as a general acid and that ratios of *cis*- and *trans*-addition of water for BP diol epoxides are very similar to the ratios obtained by hydronium ion (specific acid)-catalyzed hydrolysis; both instances suggest the intermediacy of the same benzyl carbonium ion (29).

trans-Adducts of BP *trans*-diol epoxide and DNA have been observed to predominate (30). Tetraol formation by hydrolysis of BP *trans*-diol epoxide occurs mainly with *trans*-addition of water (23). This finding of *trans*-attack is consistent with the intermediacy of a triol carbonium ion in which both *trans*-conformations hinder *cis*-addition (28). Steric hindrance by the 8-hydroxy group in the BP *cis*-triol carbonium ion would favor *cis*-addition of water, which is found experimentally (23). Thus the stereoselective pattern of BP diol epoxide hydrolysis and adduct formation with DNA could be rationalized by an intermediate carbonium ion.

Kinetic rate constants for hydrolysis of the PAH bay-region diol epoxides in acid are available (25). In water they are (M^{-1} sec^{-1}): 160 for phenanthrene *trans*-diol epoxide, 127 for chrysene *trans*-diol epoxide, and 1400 for BP *trans*-diol epoxide. Our calculated lack of activation energy for both protonated *trans*-diol epoxides of BP explains the increase by 1 order of magnitude in the acid-catalyzed hydrolysis rates relative to phenanthrene and chrysene.

The previously reported correlation of ease of carbonium ion formation with carcinogenicity is thus supported by our more sophisticated molecular orbital calculations. An additional finding is that the bay-region triol carbonium ion of BP arises spontaneously from protonation of the bay-region diol epoxides, unique to the carcinogens in the series investigated. The present work provides theoretical evidence in support of a metabolic step influential in cancer induction in which a carbonium ion is produced that is then capable of reacting with cellular constituents, including DNA, as the ultimate carcinogen of the parent hydrocarbon. However, the carcinogenicity of all PAHs cannot be explained only

on the basis of thermochemically controlled carbonium ion formation.

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