provided by a He-Ne laser. Laser intensity was varied with Corning colored glass filters and monitored with a beam splitter and a Tektronix J16 digital radiometer equipped with J6502 probe. The laser beam was masked to match the size of the Si surface.

For experiments involving switching between different solutions (e.g., with and without ferrocene), the various solutions were prepared in advance in separate Pyrex cells using a common sample of supporting electrolyte. The interchange of solutions was effected by first disconnecting the potentiostat leads and then, with all electrodes clamped in place, removing the initial cell, rinsing the electrodes in a beaker of pure solvent, and then securing the new solution-containing cell in position. The controlled addition of H_2O to ferrocene-EtOH solutions was accomplished by disconnecting the potentiostat leads and injecting by syringe a premeasured aliquot of distilled H_2O. The solution was stirred briefly by a magnetic stir bar, and the voltammetric scans were then resumed.

Electrodes destined for use in aqueous solution first had their cyclic voltammetric properties recorded in 0.1 M $[n\text{-}Bu_4N]ClO_4$-EtOH immediately after derivatization. The electrodes were then transferred to a cell containing 0.1 M $NaClO_4$ in doubly distilled deionized H_2O. For cyclic voltammetric studies (Figure 4), the electrodes were scanned several times until their voltammetric behavior had stabilized; for current vs. time studies (Figure 5), the electrodes were instead irradiated while at +0.2 V vs. SCE until the anodic current due to oxidation of surface-attached material had declined to less than 0.5 μA. Following this preliminary procedure, the $Fe(CN)_6^{4-}$ was introduced by pipet or syringe injection of a measured amount of $Fe(CN)_6^{4-}$ stock solution. In the current against time plots, mediated electron transfer to $Fe(CN)_6^{4-}$ was thereby initiated, and the photocurrent immediately assumed, and maintained, the value shown in Figure 5.

For equilibrium current-potential curves in aqueous $Fe(CN)_6^{4-}$ (Figure 7), the electrodes were derivatized and checked in 0.1 M $[n\text{-}Bu_4N]ClO_4$-EtOH as above. However, they were then transferred directly to a cell containing 0.1 M $Fe(CN)_6^{4-}$ and 0.01 M $Fe(CN)_6^{3-}$ in doubly distilled, deionized water, without preliminary cycling in 0.1 M $NaClO_4$-H_2O.

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Mechanistic Studies of Arene Oxide and Diol Epoxide Rearrangement and Hydrolysis Reactions

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Abstract: Using the semiempirical all valence electron MINDO/3 method, the mechanisms of acid-catalyzed rearrangement and hydrolysis of benzene oxide and benzene diol epoxide were studied. Both product stabilities and reaction pathways were calculated. The results show that benzene oxide prefers phenol formation to hydrolysis, and suggest that the mechanism for this reaction involves rate-determining S_N1-type formation of a carbocation quickly followed by the NIH shift, in agreement with experiment. Formation of a carbocation in benzene diol epoxide is less favorable, and should also be followed by the NIH shift leading to a ketone. Since this sort of ketone is only a minor product of the diol epoxides studied to date, it is inferred that the hydrolysis of benzene diol epoxide does not proceed by carbocation trapping, but instead by an S_N2 mechanism. These results are used to rationalize why polycyclic diol epoxides are highly mutagenic and good candidates as ultimate carcinogens, whereas polycyclic arene oxides are not.

Introduction

In 1950 Boyland suggested that arene oxides are formed as intermediates in the metabolism of polycyclic aromatic hydrocarbons.[1] Subsequent work by Jerina established that naphthalene is metabolized to 1-naphthol via an arene oxide intermediate, and demonstrated what has become the hallmark of such reactions: a Whitmore 1,2 shift[2] related to the familiar pinacol rearrangement[3] and now called the NIH shift.[4] Arene oxides have now been implicated in the formation of phenols, diols, ketones, glutathione conjugates, and other important metabolites of polycyclic aromatic hydrocarbons.[5]

Recently, much attention has been paid to the formation and reactions of arene oxides as a result of interest in the bay region hypothesis. As illustrated below, it is now thought that the activation of polycyclic aromatic hydrocarbons to ultimate carcinogens requires three steps: initial epoxidation by the P-450 monooxygenases, followed by epoxide hydrase mediated hydrolysis, and a second epoxidation. This yields a diol epoxide which can interact with tissue nucleophiles including DNA. The bay region hypothesis is supported by observations on benz[*a*]anthracene,[6] 7-methylbenz[*a*]anthracene,[7] benzo[*a*]pyrene,[8] 3-methylcholanthrene,[9] dibenz[*a,h*]anthra-

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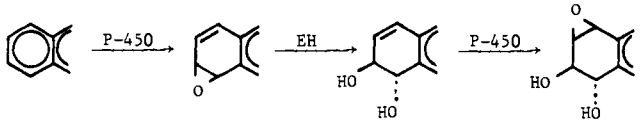
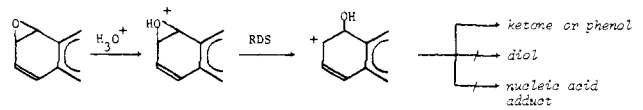
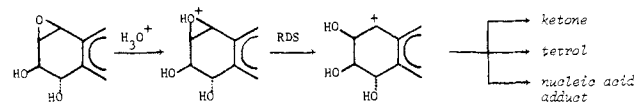


cene,[10] and chrysene,[11] all of which appear to form ultimate carcinogens by this scheme.

The electrophilicity of diol epoxides is thought by some workers to arise from their propensity to undergo specific- and general-acid-catalyzed ring opening, forming carbocations in the rate-determining step.[12,13] Such a mechanism has been proposed based on a study of the nonenzymatic hydrolysis of 7β,8α-dihydroxy-9β,10β-epoxytetrahydrobenzo[a]pyrene, the predominant diol epoxide formed and bound to DNA in mammalian P-450 preparations,[14,15] and is shown below for specific acid catalysis.



This proposed reaction mechanism for diol epoxide ring opening parallels quite closely the established mechanisms for the hydrolysis of several dihydro epoxides, including benzene oxide, naphthalene oxide, and three phenanthrene oxides.[16] Depending on the nature of the hydrocarbon and the conditions of hydrolysis, these reactions can take place either by spontaneous ring opening, via general acid catalysis, or as illustrated below via specific acid catalysis. Several lines of investigation



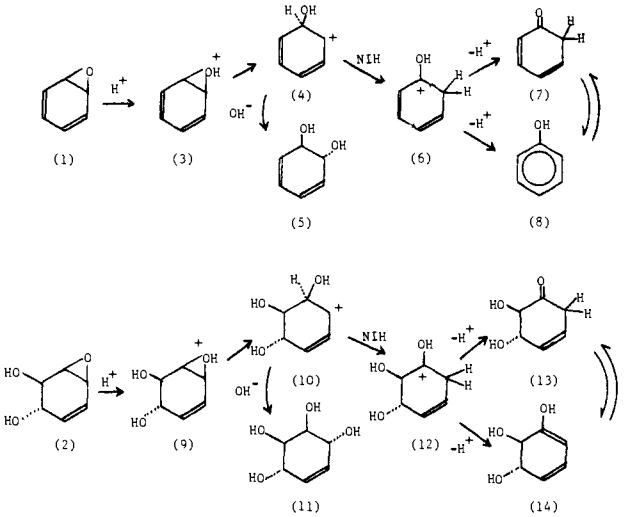
support the formation of a carbocation for both spontaneous and acid-catalyzed arene oxide ring opening, including solvent effects[17] and substituent effects.[16] Furthermore, the absence of large primary deuterium isotope effects implies that the formation of this species, and not the subsequent NIH shift, is rate determining.[16]

The similarities between the nonenzymatic reactions of dihydro epoxides and tetrahydrodiol epoxides provoke a fundamental question: why is it that diol epoxides are potent mutagens and carcinogens whereas most arene oxides require further metabolism to be more mutagenic and carcinogenic than the parent compounds? To resolve this question and better understand the mechanisms involved in the reactions of arene oxides, our laboratory has begun quantum chemical studies of the acid-catalyzed reactions of two model compounds: 1β,2β-epoxydihydrobenzene [benzene oxide (**1**)] and 1α,2β-dihydroxy-3β,4β-epoxytetrahydrobenzene [benzene diol epoxide (**2**)].

(1) (2)

Wood et al. showed that in benz[a]anthracene[18] and benzo[a]pyrene[19] the in vivo and in vitro rates of tetrahydrodiol formation from tetrahydro epoxides and tetrahydrotetrol formation from tetrahydrodiol epoxides correlate well with their mutagenic potencies. These results suggest that, for these compounds, formation of critical nucleic acid adducts proceeds by a mechanism which parallels their nonenzymatic hydrolysis.

Scheme I. Intramolecular and Intermolecular Reaction Mechanisms for the Model Compounds[a]



[a] The stereoisomers of 5 and 11 correspond to observed products of benzo[a]pyrene metabolism,[20] although other stereoisomers have been reported.[21]

Thus, only two pathways were considered in the reactions of **1** and **2**: an intramolecular pathway, leading to detoxification, and an intermolecular pathway, representing the parallel hydrolysis and nucleic acid alkylation reactions, as shown below.

Methods

Two types of calculations were made to elucidate these pathways. The first was the calculation of heats of reaction for the formation of products **5**, **7**, and **8** from **1** and the formation of **11**, **13**, and **14** from **2**. These parameters establish whether intramolecular rearrangement or attack on nucleophiles is thermodynamically preferred for each epoxide. A semiempirical all valence electron molecular orbital method, MINDO/3, parametrized to reproduce experimental heats of formation,[22] was used for these calculations. Total molecular geometry optimizations were performed on each species to ensure the internal consistency of the results.

The second set of calculations was the monitoring of the actual reaction pathways involved in proceeding from **3** through **4** to **6** and from **9** through **10** to **12**. These calculations provide a detailed description of the formation and elimination of the important carbocations **4** and **10**. Again, MINDO/3, which is surprisingly successful for such calculations,[23] was used with total geometry optimization.

It should be emphasized that, although there might well be significant errors in the energies calculated by MINDO/3, much more reliable results can be expected by examining the differences in energies of similar compounds. Thus, in interpreting the present calculations emphasis is placed on comparison between the results obtained for the epoxide and the diol epoxide.

Results

The heats of reaction show that, in the case of benzene oxide, hydrolysis is not competitive with rearrangement. Rearrangement to the ketone yields 13 kcal/mol, with tautomerization to the phenol yielding an additional 16 kcal/mol, for a total ΔH of −29 kcal/mol. These energies compare well with values recently published by Politzer and Daiker,[24] using STO-5G level ab initio methods without optimizing the product geometries. The present calculations show that hydrolysis of benzene oxide yields only 17 kcal/mol and is thus

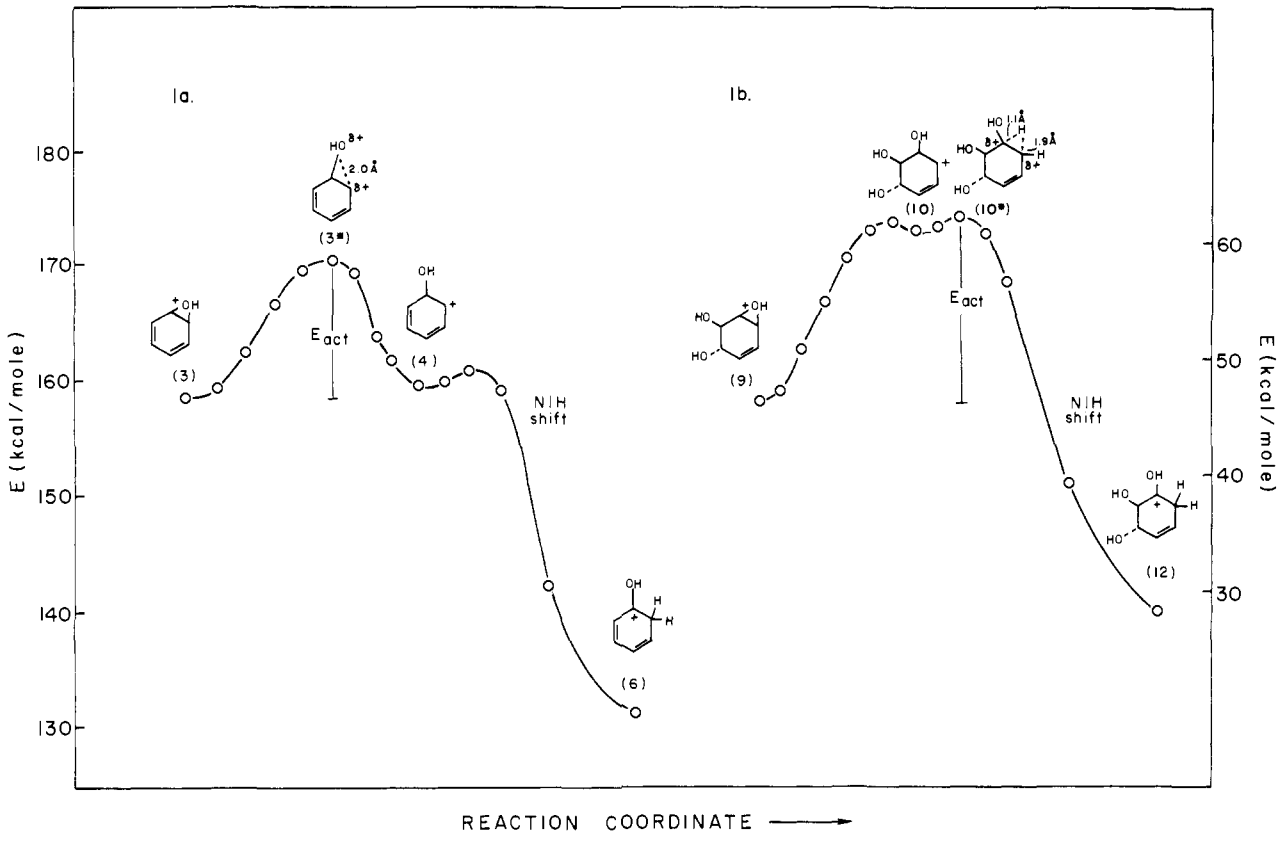
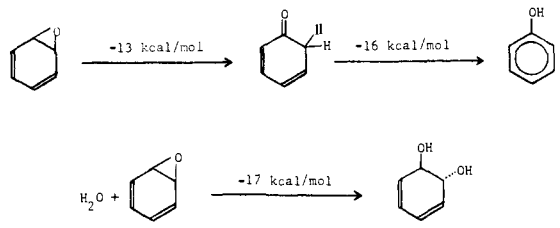
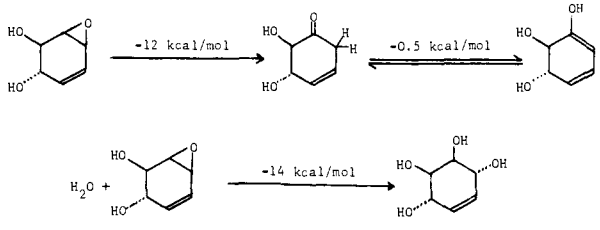


Figure 1. Reaction pathway: energy vs. reaction coordinate for (a) protonated benzene epoxide, (b) protonated benzene diol epoxide.

much less thermodynamically favorable than rearrangement.

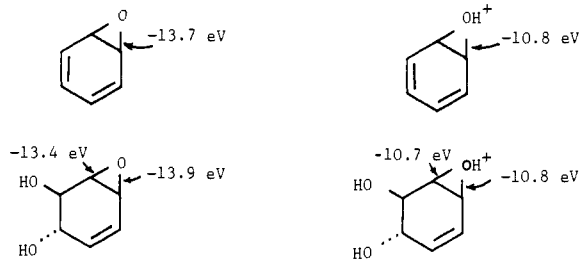


In contrast, hydrolysis of benzene diol epoxide is somewhat favored over rearrangement, as shown below. Rearrangement to the ketone yields 12 kcal/mol, comparable to the first step in the rearrangement of benzene oxide. However, tautomerization lacks the driving force of rearomatization and the enol is less than 1 kcal/mol more stable than the ketone. Therefore, hydrolysis, which leads to a product whose energy is 14 kcal/



mol lower than the reactants', is preferred.

As a preliminary step in the reaction pathway studies, the effect of protonating the epoxide oxygen on the C-O bond strength was calculated. It was verified that in both benzene epoxide and benzene diol epoxide the C-O bonds are weakened. In each case, a quantity proportional to the bond energy decreases by about 3 eV (~70 kcal/mol) as shown below. This decrease accounts for the fact that such reactions can be acid catalyzed.[16]



Reaction pathways calculated for the subsequent reaction of the protonated species show qualitative differences between benzene epoxide and benzene diol epoxide. Figure 1 summarizes the energy profiles for the two reaction pathways. The calculated geometries and energies of the species shown in this figure can be found as Tables 1-8 in the microfilm edition of this journal. In breaking the C-O bond of the protonated benzene epoxide, an activated complex (**3***) is formed with an activation energy of 12 kcal/mol. For benzene diol epoxide, more activation energy is needed to break the epoxide bond, about 16 kcal/mol. Furthermore, in benzene epoxide the intermediate carbocation (**4**) is of comparable stability to the protonated epoxide (**3**), whereas the benzene diol epoxide carbocation (**10**) is nearly 15 kcal/mol less stable than the protonated diol epoxide (**9**). Because MINDO/3 overestimates the stability of at least one epoxide, oxirane,[25] both of these energies might be exaggerated. However, a recent theoretical study of the proton-catalyzed ring opening of oxirane, using ab initio methods with a double ζ Dunning basis set, agrees qualitatively with these results.[26] They calculate an energy of activation of 25 kcal/mol, with the carbocation 7 kcal/mol less stable than the protonated epoxide. Thus, it can be concluded with some confidence that benzene epoxide has a greater tendency to form a carbocation than benzene diol epoxide.

Once formed, the carbocations of both benzene epoxide and benzene diol epoxide were found to be metastable species. In

benzene epoxide, the carbocation is a local minimum on the downslope side of the energy profile, with only 1 kcal/mol activation energy required for rearrangement via the NIH shift to **6**. This energy should be easily provided as kinetic energy gained in forming **4** from the activated complex **3***. In benzene diol epoxide, the carbocation is a local minimum on the upslope of the energy profile, with about 1 kcal/mol activation energy required for either ring closure or the NIH shift. Once again, the NIH shift leads to a thermodynamically favorable species (**12**).

Discussion

The heats of formation show that the hydrolysis of benzene epoxide is more exothermic than the hydrolysis of benzene diol epoxide. If hydrolysis and DNA attack proceed by similar mechanisms, it might be thought that arene oxides should be better candidate ultimate carcinogens than diol epoxides. However, the arene oxides also can rearomatize by intramolecular rearrangement, and this reaction was found to be far more exothermic than hydrolysis. Therefore arene oxides should have little tendency to participate in intermolecular reactions. It must be emphasized, however, that they are potentially reactive alkylating agents but that competing internal rearrangement would usually preclude such reactions. Diol epoxides might be somewhat less reactive as alkylating agents, but they have much less propensity to undergo intramolecular rearrangement.

The reaction pathway studies show that, in benzene epoxide, protonation is followed by a rate-determining epoxide bond cleavage and a rapid subsequent NIH shift, in agreement with experiment.[16] In contrast, the reaction profile for benzene diol epoxide shows that it required more activation energy and forms a less stable intermediate carbocation. Moreover, if formed, this carbocation should rearrange via the NIH shift. However, in reactions of benzo[*a*]pyrene diol epoxides, either none or small amounts of compounds like **13** are detected.[20,27] To resolve the experimental and theoretical observations, it can be hypothesized that carbocations of diol epoxides are not formed as intermediates in an S_N1-type reaction. Instead it appears that the assistance of a nucleophile in an S_N2 manner is required for their reactions with both water and nucleic acids.

In support of this hypothesis, there is evidence to implicate S_N2 mechanisms in the attack of related compounds on both water and other nucleophiles. It is known that hydrolysis of oxirane involves S_N2 attack and not carbocation trapping.[28] Furthermore, recent experimental work has shown that for the tetrahydro epoxides of phenanthrene the bay-region epoxide is more reactive to S_N2 attack by mercaptoethanol than the non-bay-region epoxide.[29] This observation suggests the possibility that the high carcinogenicity of bay-region diol epoxides arises from high S_N2 reactivity. However, there is also some evidence for S_N1 hydrolysis in benzo[*a*]pyrene diol epoxides,[13] and it is thought that bay-region diol epoxides are more reactive to S_N1 attack than non-bay-region diol epoxides.[30] Further experimental and theoretical work to determine more definitively which mechanism is involved in the reactions of diol epoxides with both water and nucleic acids could have important consequences for both our understanding of the role of these compounds in carcinogenesis and our ability to predict carcinogenic potencies.

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Supplementary Material Available: Calculated geometries and energies of the species shown in Figure 1 (5 pages). Ordering information is given on any current masthead page.

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