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Nucleophilic Displacement on the Arene Oxides of Phenanthrene

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Abstract: Phenanthrene 9,10-oxide, a K-region arene oxide, undergoes nucleophilic attack by oxygen bases and a wide variety of amines as a result of its relatively slow rate of ring opening to carbonium ion and the decreased rate of rearrangement of the carbonium ion to phenol. The rate of reaction of this oxide with nucleophiles is comparable with that of ethylene oxide. The second-order rate constants for attack by primary and secondary amines give a β_{nuc} value of 0.4. Certain tertiary amines (trimethylamine and quinuclidine amines) exhibit enhanced nucleophilic reactivity. This is suggested to be due to preequilibrium complex formation prior to nucleophilic attack. The quinuclidine amines give a β_{nuc} value of 0.1. Oxygen bases are less reactive nucleophiles and give a β_{nuc} value of 0.2. In the case of non-K-region arene oxides (phenanthrene 1,2-oxide, phenanthrene 3,4-oxide, benzene oxide, and naphthalene oxide), nucleophilic attack by amines and oxygen bases is not sufficiently rapid to compete with the spontaneous aromatization reaction. Thiolate anions exhibit considerably greater nucleophilic reactivity than do amines and, consequently, react with both the K-region and non-K-region arene oxides. The second-order rate constants for attack by thiolate anions result in a β_{nuc} value of 0.2 for each of the arene oxides investigated. Glutathione is no more reactive a thiol than would be predicted from its pK_a. NMR studies of the nucleophilic adducts show that nucleophilic attack is stereospecific and results in trans adducts. Thus either direct SN2 attack or nucleophilic trapping of tight ion pairs is occurring. An index of nucleophilic susceptibility is defined for epoxides and arene oxides: ethylene oxide = 1, phenanthrene 9,10-oxide = 0.3, and the non-K-region arene oxides are all <0.01.

Introduction

During the normal course of metabolism of aromatic compounds, arene oxides occur as intermediates.[2] For quite some time certain arene oxides generated from xenobiotic hydrocarbons have been considered to be carcinogenic.[3] It is currently thought that the carcinogenic behavior may be the result of covalent binding of the arene oxide to proteins and nucleic acids.[4] Consequently, studies of the reactivity of arene oxides toward nucleophilic attack[5a] have been carried out to determine the feasibility of this hypothesis.[5a,b] The results of these studies have shown that while polarizable nucleophiles such as thiols and azide readily attack arene oxides, nonpolarizable nucleophiles such as amines and hydroxide ion show no reactivity. In the previous paper[6] we have shown, in a study of the comparative mechanisms of solvolysis of the K-region and non-K-region arene oxides of phenanthrene, that the major product of solvolysis of the K-region arene oxide in neutral and basic solution is the trans diol while under the same conditions the non-K-region arene oxides aromatize exclusively to phenols. The K-region arene oxide readily undergoes nucleophilic attack by hydroxide ion and water and thus appears to be more epoxide-like in its reactivity than the non-K-region oxides. Since it is the K-region arene oxides that show carcinogenic activity[7] and bind covalently to cellular constituents,[8] and since previous studies of nucleophilic reactivity have been carried out only on benzene oxide and other simple non-K-region arene oxides, it is apparent that an investigation of the susceptibility of a K-region oxide to nucleophilic attack is necessary. In the present study, we have undertaken an investigation of the reactivity of nitrogen, oxygen, and sulfur nucleophiles on phenanthrene 9,10-oxide, the K-region arene oxide of phenanthrene, and for comparative purposes on phenanthrene 1,2-oxide and phenanthrene 3,4-oxide, the isomeric non-K-region arene oxides. The kinetics of the reaction have been studied and the stereochemistry of the products established.

Experimental Section

Materials. The preparations of the arene oxides used in the present study have been described previously.[6] The hydrochlorides of me-

thylamine, dimethylamine, ethylamine, glycinamide, quinuclidine, 3-quinuclidinone (Aldrich), 3-chloroquinuclidine, trifluoroethylamine, and hydrazine were recrystallized from water–ethanol. Methoxyamine hydrochloride and 2-mercaptoethylamine hydrochloride (Sigma) were recrystallized from ethanol–ether and imidazole from acetone–petroleum ether. Sodium thioglycolate (Sigma), 2-mercaptoethanol (Sigma), glutathione (National Biochemical), 3-quinuclidinol (Aldrich), and glycylglycine were used without further purification. Allylamine, pyrrolidine, piperidine, benzylamine, pyridine, *n*-butylamine, and *tert*-butylamine were distilled just prior to use. All solids were stored in a vacuum desiccator over P_2O_5.

Kinetic Measurements. All kinetic determinations were done in aqueous solution containing 10^{-4} M EDTA to scavenge extraneous metal ions and at a temperature of 30 °C with the ionic strength maintained at 1.0 with KCl. Fresh doubly glass-distilled water was used to make up all kinetic solutions. The concentration of arene oxide employed in the kinetic studies was $\sim 1 \times 10^{-5}$ M. The rates of nucleophilic addition were determined on either a Cary 15, Cary 16, Cary 118, or a Gilford Model 2000 spectrophotometer. All spectrophotometers were thermostated at 30 °C. Amine addition to phenanthrene 9,10-oxide was followed by monitoring the appearance of product at 255 or 275 nm. Reaction of amines with phenanthrene 1,2- and phenanthrene 3,4-oxide was investigated at 240 and 265 nm, respectively. Because of the absorption of thiolate anion, the rate of addition of this species was followed at 285 nm. All reactions were carried out under a blanket of argon or nitrogen in standard tapered cuvettes.

The buffer solutions were prepared immediately prior to use by the addition of standardized KOH or HCl to the acid or base form of the buffer. When possible reactions with amines and thiols were studied at a pH = $pK_a \pm 1$; thus the amine or thiol acted as its own buffer. From three to five serially diluted buffer solutions were employed at each pH. In the case of weakly basic amines and carbonate ion, the concentration range employed was 0.1–0.5 M. Because of their greater reactivity, a concentration range of 0.02–0.1 M was generally employed with thiols and strongly basic amines. The pH's of the serial dilutions agreed within 0.02 pH unit. Readings of pH were determined on a Radiometer Type PMH 26 pH meter. Aminolysis reactions carried out at a pH beyond the buffering capacity of the amine were monitored in a Radiometer pH-stat assembly specifically designed for a Cary 15 spectrophotometer.[9]

For the reactions with thiols, all operations in the preparation of the buffers were performed in Halzene plastic wear. Cuvettes were soaked in 10^{-3} M EDTA for 24 h prior to use. In order to remove heavy metal ions, the solution of KCl employed in the buffer preparation was shaken with an equal volume of a 0.01% solution of dithiazone in CCl_4. The dithiazone was removed by repeated extraction with CCl_4 (until all color had disappeared), and the residual CCl_4 was removed by bubbling with argon.

Calculations of the pseudo-first-order rate constants and least-squares slopes and intercepts were done using a Hewlett-Packard Model 9820A computer or the UCSB on-line interface to an IBM 360-75 computer.

pK_a Determinations. The pK_a's of the amines were determined by half-neutralization. The microscopic pK_a's of the thiols are those previously determined in this laboratory.[5]

Product Studies. The structures of the nucleophilic adducts obtained from reaction of phenanthrene 9,10-oxide with a variety of nucleophiles were examined after reaction had gone to completion both in water under the conditions of the kinetic studies and in aqueous tetrahydrofuran. Products were isolated by differential extraction into organic solvent (methylene chloride or ethyl acetate), the solvent was dried (Na_2SO_4), and the desired products were examined by nuclear magnetic resonance after removal of the extraction solvent. All of the adducts gave acceptable mass spectra either before or after acetylation of the free hydroxyl group. Acetylation was conducted by storing the adducts in pyridine:acetic anhydride (1:3) for 24 h at room temperature, followed by conventional workup. For reactions run in water, the solutions were saturated with NaCl prior to extraction.

A. Under Kinetic Conditions. For reaction with 2-mercaptoethanol, a homogeneous solution of 15.6 g of thiol, 50 ml of 1.0 M KOH, 20 ml of 0.1 M EDTA, 1948 ml of 1.0 M KCl, and 40 mg of phenanthrene 9,10-oxide in 80 ml of acetonitrile was agitated at 30 °C for 15 min. Products were extracted into methylene chloride. Residual 2-mercaptoethanol in the concentrated extract was removed under high vacuum at 70 °C. For reaction with thiolacetic acid, a solution of 0.10 g of thiolacetic acid, 20 mg of phenanthrene 9,10-oxide in 40 ml of acetonitrile, and 475 ml of phosphate buffer (0.1 M, pH 7.0) was agitated at 30 °C overnight. The methylene chloride extract was washed with 1% $NaHCO_3$ to remove residual thiolacetic acid prior to final work up. For reaction with dimethylamine, a solution of 28.6 g of dimethylamine hydrochloride, 175 ml of 1 N KOH, 525 ml of 1 M KCL, 20 mg of phenanthrene 9,10-oxide in 40 ml of acetonitrile, and sufficient water to bring the final volume to 3.5 l. was agitated at 30 °C for 40 h. The initial and final pH was 10.7. Residual dimethylamine was lost on concentration of the methylene chloride extract.

B. In Aqueous Tetrahydrofuran. Reactions of phenanthrene 9,10-oxide with nucleophiles were also run at high concentration in small volumes of an aqueous organic solvent to facilitate isolation. As will be shown later, changing the solvent from water to aqueous tetrahydrofuran gave no change in product composition with dimethylamine as the nucleophile. Reactions were monitored by TLC and run until the arene oxide had been consumed. Reaction with azide was conducted in refluxing ethanol for 3 h.[10] Reaction with methylamine, dimethylamine, *n*-butylamine, and *tert*-butylamine were conducted in 50–60% aqueous THF and were run at room temperature for 2 days, 3 h, 24 h, and for 2.5 h at reflux, respectively, in the presence of a large excess of the amine. The reaction with *n*-butylamine is typical; a solution of 40 mg of phenanthrene 9,10-oxide, 2 ml of amine, and 12 ml of 50% water in THF (homogeneous) was stored at room temperature for 24 h. Products were extracted into ethyl acetate. The presence of water in the solvent greatly enhanced the rate of reaction. The *n*-butylamine adduct had previously been prepared by refluxing the arene oxide in the amine.[10] For reaction with methoxide, a suspension of 40 mg of phenanthrene 9,10-oxide was stirred with 12 ml of 6% sodium methoxide in methanol for 24 h.

Results

Reactions of amines, oxygen bases, and thiols with phenanthrene 9,10-oxide were carried out under conditions such that the concentration of the nucleophile was much larger than the concentration of oxide. Thus, pseudo-first-order kinetics were obtained. The observed rate constants are generally well correlated by the rate expression

$$k_{obsd} = k_{ly} + k_n \frac{K_a}{(K_a + a_H)} [N]_T \qquad (1)$$

where k_{ly} is the rate constant for reaction of the arene oxide with lyate species (H_3O^+, H_2O, HO^-) at the pH at which the nucleophilic reaction is carried out, k_n is the second-order rate constant for nucleophilic attack, $[N]_T$ is the total concentration of nucleophile and its conjugate acid, and $K_a/(K_a + a_H)$ is the mole fraction of the nucleophile present in the basic form. In the case of aminolysis reactions, $[N]_T = [N] + [^+NH]$, and for reaction with thiols, $[N]_T = [RS^-] + [RSH]$. The term K_a represents the acid dissociation constant of ^+NH or RSH, and a_H the hydrogen ion activity as determined by the glass electrode.

For the reaction of glycinamide with phenanthrene 9,10-oxide, a plot of k_{obsd} vs. total amine concentration is shown in Figure 1. From the figure it is obvious that the reactive species is the free base form of the amine. A similar plot may be found in Figure 2 for the reaction of phenanthrene 9,10-oxide with a thiolate anion. The second-order nucleophilic rate constants (k_n) were obtained by dividing the slopes of these buffer dilution plots by $K_a/(K_a + a_H)$ (eq 1). In some cases, curved buffer dilution plots were obtained from the reaction of tertiary amines with phenanthrene 9,10-oxide, such as that given in Figure 3 for 3-quinuclidinol. The slopes of such plots were determined using only the rates at the lower buffer concentrations.

In the previous paper, the solvolysis of phenanthrene 9,10-oxide was shown to be catalyzed by general acids.[6] Since the protonated amine may act as a general acid, the rate expression of eq 1 is more correctly expressed as

$$k_{obsd} = k_{ly} + k_{HA} \frac{a_H}{K_a + a_H} [N]_T + k_n \frac{K_a}{K_a + a_H} [N]_T \qquad (2)$$

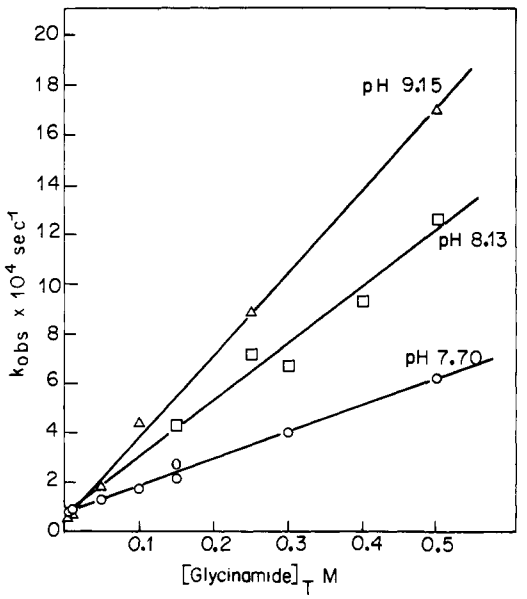


Figure 1. Plots of the observed first-order rate constants for the reaction of glycinamide with phenanthrene 9,10-oxide vs. the total concentration of glycinamide at several hydrogen ion concentrations.

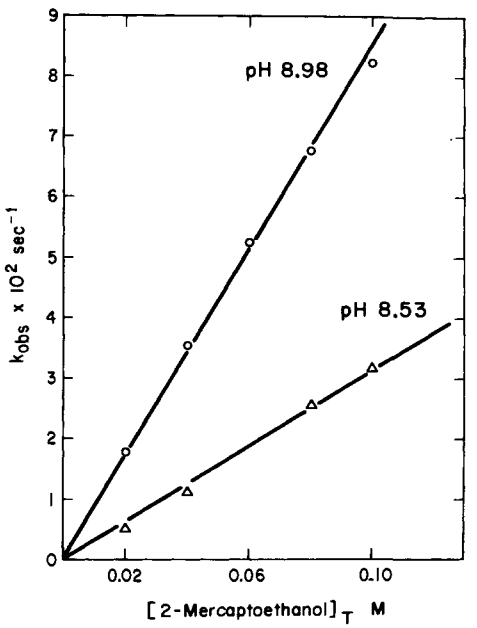


Figure 2. Plots of the observed first-order rate constants for the reaction of 2-mercaptoethanol with phenanthrene 9,10-oxide vs. the total concentration of 2-mercaptoethanol at two hydrogen ion concentrations.

For all the amines investigated in this study, with the exception of Tris, $k_n \gg k_{HA}$; thus eq 2 becomes eq 1, and values of k_n can be obtained from the slopes of buffer dilution plots as described above. Tris, however, is less efficient a nucleophile than would be predicted from its pK_a (see Figure 6), most likely because of steric factors. Consequently, k_n is only slightly greater than k_{HA}; thus in addition to the nucleophilic reaction given by the free base form of the amine, the protonated amine acts as a general acid in the solvolysis reaction. The slopes of such buffer dilution plots (Figure 4) are equal to

$$k_{HA}\frac{a_H}{K_a + a_H} + k_n\frac{K_a}{K_a + a_H}$$

Values of k_n and k_{HA} can be obtained from the slopes and intercepts, respectively, of secondary plots of slope/a_H/(K_a +

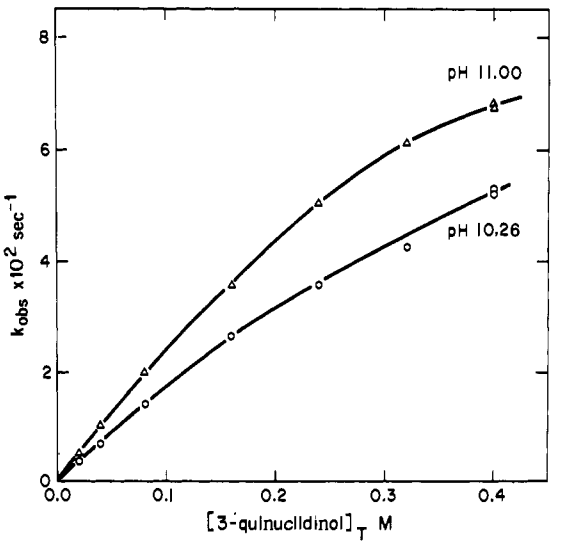


Figure 3. Plots of the observed first-order rate constants for the reaction of 3-quinuclidinol with phenanthrene 9,10-oxide vs. the total concentration of 3-quinuclidinol at two hydrogen ion concentrations.

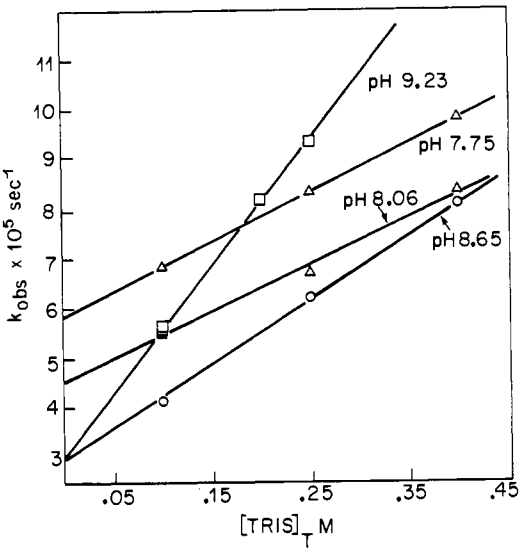


Figure 4. Plots of the observed first-order rate constants for the reaction of Tris with phenanthrene 9,10-oxide vs. the total concentration of Tris at several hydrogen ion concentrations.

a_H) vs. K_a/a_H. The secondary plot for Tris is given in Figure 5.

In the case of hydrazine and the weakly basic amines, methoxyamine and pyridine, the nucleophilic reactions were followed at a pH beyond the buffering capacity of the amine where the fraction of N-protonated amine $[a_H/(K_a + a_H)]$ is essentially zero. For this purpose buffering was accomplished with a pH-stat. The second-order rate constants for these nucleophiles were calculated from the equation

$$k_n = \frac{k_{obsd} - k_{ly}}{\frac{K_a}{K_a + a_H}[N]_T} \quad (3)$$

where k_{ly} is the hydrolytic rate constant at the pH at which the nucleophilic reaction is carried out.[6] Values of k_n for the reaction of amines and carbonate ion with phenanthrene 9,10-oxide are listed in Table I together with the pK_a's of the nucleophiles. The rate constants for nucleophilic attack by water and hydroxide ion given in the Table were obtained from the preceding paper.[6]

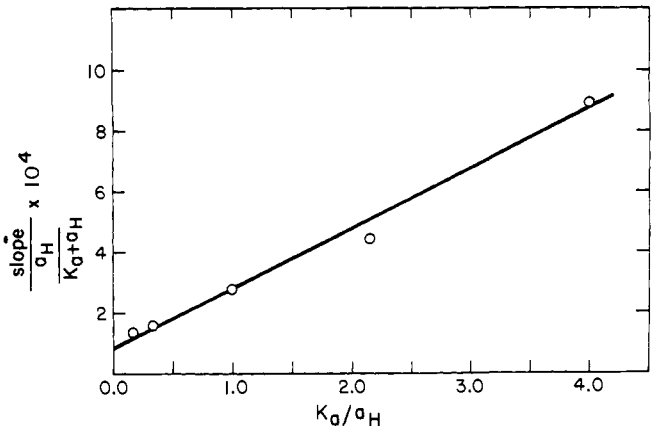


Figure 5. The slopes derived from k_{obsd} vs. $[Tris]_T$ plots divided by the fraction of the buffer present in the acid form plotted against the acid ionization constant of Tris divided by the hydrogen ion activity.

A second-order rate constant of 5.58×10^{-3} M^{-1} s^{-1} was obtained for the reaction of *N*-methylpiperidine with phenanthrene 9,10-oxide in D_2O. From this value together with the nucleophilic rate constant for that amine in water given in Table I, a deuterium solvent kinetic isotope effect (k_H/k_D) of 0.9 can be calculated.

Rate constants for the reaction of phenanthrene 1,2-oxide and phenanthrene 3,4-oxide were determined in the presence of varying concentrations (0.02–0.10 M) of pyrrolidine, the most basic of the amine nucleophiles employed for reaction with phenanthrene 9,10-oxide. No enhancement in the rate of disappearance of these oxides over that expected for spontaneous aromatization[6] was observed in the presence of the amine.

The second-order rate constants for the reaction of thiolate anions with phenanthrene 1,2-oxide, phenanthrene 3,4-oxide, and phenanthrene 9,10-oxide are given in Table II. The reaction of 2-mercaptoethanol with naphthalene oxide resulted in a second-order rate constant of 1.69 M^{-1} s^{-1} for attack by the thiolate anion.

Attempts were made to investigate the reaction of phenanthrene 9,10-oxide with proteins and nucleotides. Both protein and nucleotide absorption, however, obscured all wavelengths where disappearance or appearance of products could be monitored.

Pertinent aspects of the nuclear magnetic resonance spectra for the nucleophilic adducts of phenanthrene 9,10-oxide prepared in water and in aqueous tetrahydrofuran, as well as the acetate derivatives, are summarized in Table III. All reactions whose products were examined by NMR went to completion and gave single products with the following exceptions: (1) reaction with azide produced a minor (30% judged by NMR) nonphenolic product, with high R_f on TLC compared to the adduct, which was assumed to be 9-azidophenanthrene resulting from dehydration and (2) reaction with 2-mercaptoethanol in water proceeded to ~50% completion and attempted acetylation of the product resulted in aromatization. Although the adducts formed with azide and *n*-butylamine had been reported,[10] no spectral proof was presented to support the suggestion that these compounds had resulted from direct trans opening of the arene oxide. The spectra of the products obtained from reaction of dimethylamine with phenanthrene 9,10-oxide in water and in 50% THF in water are identical. Reaction of 3-bromophenanthrene 9,10-oxide with dimethylamine was examined in water and in 50% THF in water. In both solvents two products are formed. They appear to be trans isomers (ratio 55:45) and differ with respect to point of attack by the amine on this unsymmetrical arene oxide. The spectral evidence, combined with TLC and high-pressure liquid chromatography, indicate that all of the nucleophilic addition reactions occur by direct trans opening of the oxirane ring.

Table I. Second-Order Rate Constants for the Reaction of Nucleophiles with Phenanthrene 9,10-Oxide

Nucleophile	pK_a[a]	k_n, M^{-1} s^{-1}
Hydroxide ion	15.75	4.73×10^{-4}
CO_3^{2-}	9.68	4.27×10^{-5}
H_2O	−1.74	4.47×10^{-7}
Pyrrolidine	11.44	6.33×10^{-2}
Quinuclidine	11.22	4.41×10^{-1}
Piperidine	11.03	5.96×10^{-2}
Dimethylamine	11.00	7.43×10^{-2}
Methylamine	10.82	2.41×10^{-2}
Ethylamine	10.73	6.48×10^{-3}
3-Quinuclidinol	10.26	3.51×10^{-1}
N-Methylpiperidine	10.23	5.00×10^{-3}
Trimethylamine	9.95	1.05×10^{-1}
Allylamine	9.92	6.67×10^{-3}
Benzylamine	9.50	9.63×10^{-3}
L-Lysine	9.07	7.31×10^{-3}
N-Methyldiethanolamine	8.88	1.16×10^{-3}
3-Chloroquinuclidine	8.83	2.13×10^{-1}
Tris	8.25	2.77×10^{-4}
Hydrazine	8.18	7.94×10^{-3}
Glycylglycine	8.16	3.43×10^{-3}
Glycinamide	8.13	3.61×10^{-3}
3-Quinuclidinone	7.32	1.12×10^{-1}
Imidazole	7.14	7.14×10^{-4}
Pyridine	5.30	3.00×10^{-3}
Methoxyamine	4.62	5.50×10^{-4}

[a] Amine pK_a's were determined by half-neutralization at 30 °C in water (μ = 1.0).

Table II. Second-Order Rate Constants for the Reaction of Thiolate Anions with Phenanthrene Oxides

		k_n, M^{-1} s^{-1}		
Thiol	pK_a[a]	9,10-Oxide	1,2-Oxide	3,4-Oxide
Sodium thioglycolate	9.82	3.76	2.66	2.19
2-Mercaptoethanol	9.45	3.38	1.58	2.04
Glutathione	8.72	2.31	1.00	1.31
2-Mercaptoethylamine	8.22	1.83	1.16	1.46

[a] pK_a's obtained from ref 5b.

Discussion

Reaction with Oxygen Bases and Primary and Secondary Amines. To date, only soft polarizable nucleophiles such as thiolate and azide anions have been found to exhibit high nucleophilic reactivity towards benzene and naphthalene oxides. Hydroperoxide, phenoxide, and alcoholates react more slowly and require nonaqueous solvent. Amines, hydroxide ion, and water exhibit no nucleophilic reactivity.[5a,b] In agreement with these findings, the non-K-region oxides of phenanthrene (phenanthrene 1,2-oxide and phenanthrene 3,4-oxide) exhibit no reactivity with amines or water and no reactivity with hydroxide ion up to pH 14. The observed rate constant in the presence of these nucleophiles is simply that for spontaneous aromatization to phenanthrols.

Phenanthrene 9,10-oxide with the epoxide ring at the K-region position reacts with water, carbonate ion, and hydroxide ion,[6] as well as with primary, secondary, and tertiary amines. A Bronsted plot for the reaction of oxygen bases with the arene oxide is given in Figure 6; the slope of β_{nuc} is 0.2. As has been found with ethylene oxide,[11] amines exhibit greater nucleophilic reactivity toward the arene oxide than do oxygen bases. Bronsted plots for amine nucleophilic attack are given in Figure 7. Primary and secondary amines, with few exceptions, fall on a line whose slope gives a Bronsted β_{nuc} value of 0.4. The negative deviation evidenced by Tris is likely due to the bulky

Table III. Chemical Shifts (δ, Relative to Internal Me_4Si in $CDCl_3$) and Coupling Constants ($^3J_{9,10}$) for Nucleophilic Adducts at the K-region of Phenanthrene 9,10-Oxide Measured at 100 MHz after Exchange with Methanol-d [a]

Nucleophile (Nu) or Compd	Adduct			Acetate of adduct		
	OD / H	Nu / H	J	OAc / H	NuAc / H	J
CH_3O^-	4.87	4.38	8.4	6.11	4.37	4.6
trans-Dihydrodiol monoacetate [b]			8.2			
cis-Dihydrodiol monoacetate [b]			3.8			
$HOCH_2CH_2S^-$	4.82	4.24	3.9			
$CH_3C(=O)S^-$	4.79	5.11	3.5	5.99	5.13	2.7
Trans adduct from $CH_3C(=O)S^-$ on benzene oxide [c]			3.5			2.0
CH_3NH_2	4.64	3.66	7.2		Complex [d]	~4
$(CH_3)_2NH$	4.81	3.70	6.2	6.20	3.67	3.4
$(CH_3)_2NH$ on 3-bromophenanthrene 9,10-oxide (two products) [e]	4.77	3.67	6.0	6.15	3.66	3.2
	4.83	3.69	5.5	6.19	3.66	3.2
tert-Butylamine	4.34	3.81	9.4	5.88	4.07 [f]	3.6
n-Butylamine	4.59	3.72	8.0		Complex [g]	~3
N_3^-	4.75 [h]	4.61	7.2	5.98	4.70	5.2
trans-Halohydrin acetate of 9,10-dihydrophenanthrene [i]						3.4

[a] Only data at the 9,10 position are given. [b] Data taken from H. Selander, H. Yagi, D. M. Jerina, M. C. Wells, J. F. Davey, V. Mahadevan, and D. T. Gibson, submitted for publication. [c] Data taken from ref 4. [d] The adduct acetylates at both the hydroxyl and the methylamino functional groups. The spectrum was run at 80 °C in benzene. At lower temperatures in benzene-d_6 or $CDCl_3$ a very complex spectrum is observed due to restricted rotation and magnetic anisotropy of the amide group; see H. Paulsen and K. Todt, *Angew. Chem. Ed. Eng.*, **5**, 899 (1966) for related examples and the *n*-butylamine adduct in the table. [e] Both adducts are trans and differ with respect to point of attack of the amine on the unsymmetrical arene oxide. [f] The amino group in this compound is not acetylated. [g] The adduct acetylates at both the hydroxyl and amino groups. Room temperature spectra in $CDCl_3$ or benzene-d_6 are complex. At 80 °C in benzene-d_6, the signal for $-N(Ac)Bu$ has become so broad that it is no longer visible while signal for $-OAc$ appears as a sharp doublet at δ 6.3 with $J \sim 3$ Hz. [h] Assignment as to which carbon atom has the nitrogen substituent is not absolute. The signal at δ 4.75 has been assigned to that with the OD substituent as it is somewhat broader than that at δ 4.61. [i] Data taken from P. Dansette and D. M. Jerina, *J. Am. Chem. Soc.*, **96**, 1224 (1974).

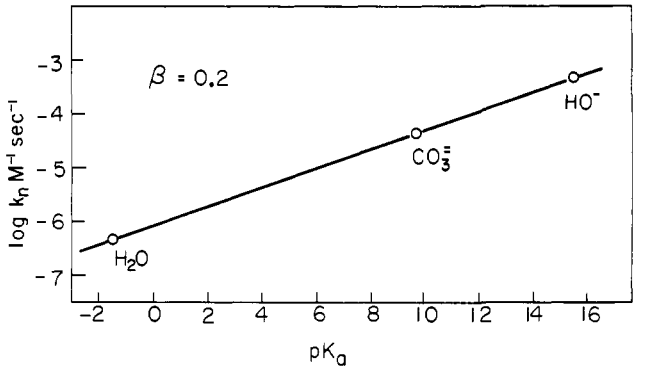


Figure 6. Bronsted plot of the second-order rate constants for the reaction of oxygen nucleophiles with phenanthrene 9,10-oxide.

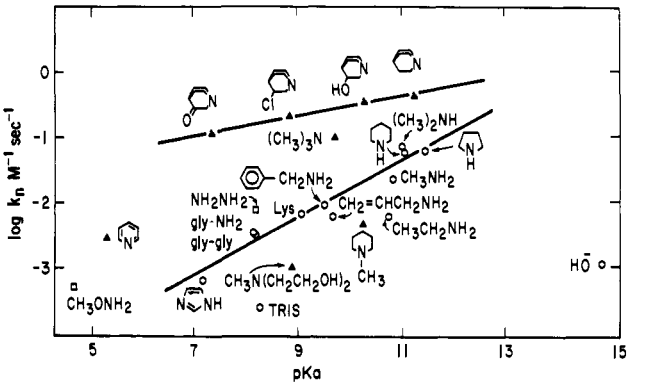


Figure 7. Bronsted plots of the second-order rate constants for the reaction of primary and secondary amines (○) and tertiary amines (▲) with phenanthrene 9,10-oxide at 30 °C vs. the pK_a of the nucleophile. The points for α-effect amines (□) and hydroxide ion are included.

substituents on the amino group.[12] Ethylamine is also somewhat less reactive than its pK_a would predict. The reason for this is not obvious, but ethylamine shows a negative deviation of approximately the same magnitude in a Bronsted plot for the attack of nucleophiles on ethylene oxide.[11]

The large negative deviation exhibited by hydroxide ion and the positive deviations of the α-effect nucleophiles, methoxyamine and hydrazine, exhibited in Figure 7 are also characteristic of the reaction of these nucleophiles with ethylene oxide. Virtanen and Korhonen[11] report that the reaction of amine nucleophiles with ethylene oxide in water gives rise to a curved Bronsted plot, i.e., one that shows a decreasing dependence of the rate of nucleophilic attack with increasing amine basicity. The amines, however, that give rise to the curvature are ethylamine, diethylamine, and triethylamine. Drawing a curved Bronsted plot through the points for these nucleophiles results in a substantial positive deviation for piperidine, the only other amine investigated of pK_a greater than 10. Because of the negative deviations obtained by us for the reaction of ethylamine and sterically hindered tertiary amines with phenanthrene 9,10-oxide, there is reason to exclude these amines from a calculation of the Bronsted slope. If this is done, the second-order rate constants for the reaction of amines with ethylene oxide fall on a straight line and result in a β_{nuc} value of 0.3.

A means for comparing the susceptibility of two substrates toward nucleophilic attack is to plot log k_n for the reaction of nucleophiles with one substrate vs. like values for a second substrate.[12a,13] A plot of this type normalizes the scattering of points due to steric effects, electronic effects, and the α effect. Such a plot is given in Figure 8 for the reaction of HO^-, H_2O, primary, secondary, and tertiary amines with phenanthrene 9,10-oxide (30 °C) and ethylene oxide (25 °C).[11] The line drawn through the experimental points of Figure 8 is generated from the equation

$$\log k_{n(\text{phenanthrene 9,10-oxide})} = 1.3 \log k_{n(\text{ethylene oxide})} + 1.7 \quad (4)$$

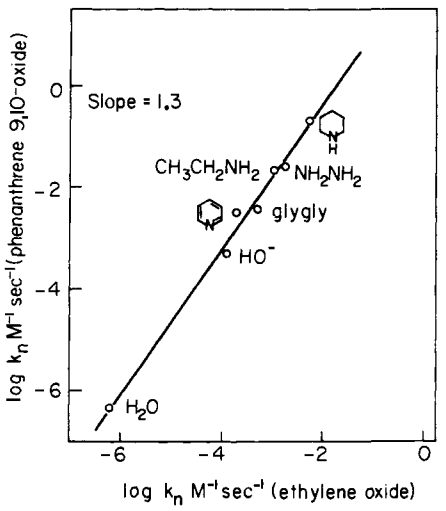


Figure 8. A plot of the log of the second-order rate constants for reaction of nucleophiles with phenanthrene 9,10-oxide vs. like values for reaction with ethylene oxide.

The plot in Figure 8 has a slope of 1.3, indicating, as do the β_{nuc} values, that the susceptibility of the arene oxide toward nucleophilic attack is somewhat more sensitive than ethylene oxide to the basicity of the attacking nucleophile. The most important conclusion to be gained from Figure 8 is that the K-region arene oxide acts very much like a normal epoxide in its reaction with nucleophiles.

The mechanism by which nucleophiles attack aliphatic epoxides has been extensively studied.[14] The ring-opening reaction is dependent on the concentration of the nucleophile, yields products obtained by attack on the least sterically hindered carbon atom rather than on the carbon atom that would give the most stable carbonium ion, and gives Walden inverted products. Thus the preferred mechanism is direct nucleophilic attack on the epoxide ring, i.e., a classical SN2 mechanism. In the case of nucleophilic attack on phenanthrene 9,10-oxide, the rate is dependent on the concentration of nucleophile (Figures 1 and 2) and, depending on the basicity of the nucleophile, is as much as 10^3-fold faster than the rate of general acid catalyzed ring opening to carbonium ion with water acting as the general acid. Thus, the reaction of nucleophiles with arene oxides also appears to proceed through a direct nucleophilic displacement mechanism (SN2) rather than via a carbonium ion trapping mechanism (SN1).[5b]

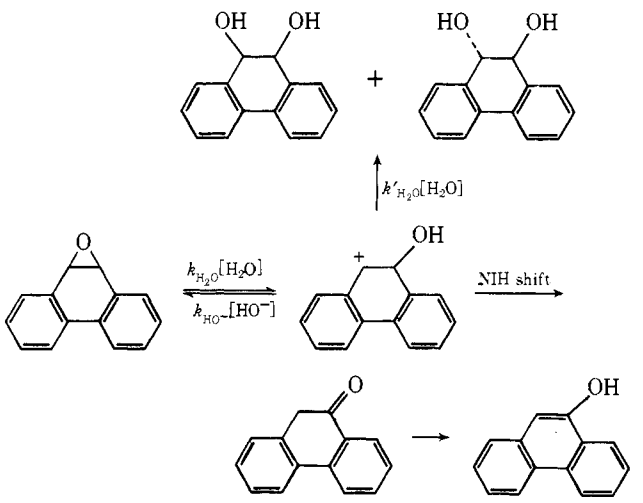
An alternate mechanism to explain reactions that in the past have been ascribed to SN2 type mechanisms is nucleophilic displacement via an intimate ion pair intermediate[15]

$$R\text{-}X \rightleftharpoons [R^+X^-] \xrightarrow{k[N]} R\text{-}N + X^- \qquad (5)$$

Since the ion pair is in reality an "extended covalent bond with considerable ionic character", it maintains its stereochemical integrity, making a choice between the two mechanisms difficult except under certain circumstances. The data plotted in Figure 8 suggest that ethylene oxide and phenanthrene 9,10-oxide follow the same mechanism in their reaction with hydroxide ion, water, and amines. A choice cannot be made between the classical SN2 and intimate ion pair mechanisms on the basis of the relative magnitude of the rate constants for nucleophilic attack on ethylene oxide vs. phenanthrene 9,10-oxide since the resonance stabilization associated with the phenanthrene oxide ion pair would have opposing effects on the reaction rate; i.e., resonance stabilization would favor the equilibrium constant for formation of the ion pair but would disfavor nucleophilic attack. The two mechanisms also cannot be differentiated on the basis of the β_{nuc} values. The relatively small β_{nuc} values (0.4 for phenanthrene 9,10-oxide and 0.3 for ethylene oxide) obtained for the reaction of primary and secondary amines are in accord with little bond formation in the transition state. This feature could be interpreted as implicating either a starting material like transition state, or attack upon the positive pole of an intimate ion pair. That the transition state for the classical SN2 reaction might be expected to resemble starting materials more than products is to be anticipated on the basis that the reaction is exothermic (Hammond postulate).[16] Both mechanisms, therefore, would be expected to be associated with small values of β_{nuc}.

In order to answer the question of why the K-region arene oxide of phenanthrene is subject to nucleophilic attack by amines and by oxygen bases while the isomeric non-K-region arene oxides do not show such reactivity, one must compare the "spontaneous" reaction rates. Opening of the epoxide ring to a carbonium ion takes place with an observed rate constant of 2.1×10^{-4} s^{-1} in the case of the K-region arene oxide. The corresponding rate constants for phenanthrene 1,2-oxide and phenanthrene 3,4-oxide are 3.10×10^{-2} and 5.55×10^{-2} s^{-1}, respectively.[6] Thus, the decreased stability of the carbonium ion from the 9,10-oxide as compared with the stability of the isomeric carbonium ions results in a greater than 100-fold decrease in the rate of the ring-opening reaction. Furthermore, formation of carbonium ion from the 1,2- and 3,4-oxides is followed by a rapid NIH shift and aromatization to give solely phenolic products. In the case of the K-region arene oxide, the rate of the NIH shift is repressed since the carbonium ion, although less stable, is subject to the competing reactions of solvent trapping to give the cis and trans 9,10-dihydro diols and hydroxide ion-catalyzed reclosure back to arene oxide (Scheme I).[6] The combined effects of the decreased rate of carbonium

Scheme I



ion formation and decreased rate of NIH shift are responsible for the existence of the K-region arene oxide in solution long enough to be attacked by nucleophiles. The decreased rate of ring opening alone is sufficient to account for the reaction of the arene oxide with all but the weakest nucleophiles employed in this study. The additional advantage of the depressed rate of the NIH shift makes possible the reaction of the arene oxide with nucleophiles of a wide variety of activity.

Reaction with Tertiary Amines. Because of the widely varying steric requirements of tertiary amines, they are not expected to give good linear free-energy correlations. However, the scatter observed in the points of Figure 7 representing the reaction of tertiary amines with phenanthrene 9,10-oxide cannot be attributed solely to steric factors; different mechanistic behavior must be involved. The negative deviations exhibited by *N*-methylpiperidine and *N*-methyldiethanolamine

are typical of the behavior of tertiary amines in Bronsted plots because of the greater steric hindrance associated with these amines.[17] The deuterium solvent kinetic isotope effect (k_{H_2O}/k_{D_2O}) of 0.9 obtained for the reaction of phenanthrene 9,10-oxide with *N*-methylpiperidine indicates that the tertiary amine is acting directly as a nucleophile and not as a general base for catalysis of attack by water.

What is surprising in Figure 7 are the rather large positive deviations evidenced by trimethylamine, quinuclidine, and substituted quinuclidines. Trimethylamine is considerably more reactive a nucleophile than is methylamine. It may be that the geometry of these tertiary amines is such to allow rapid formation of a preequilibrium complex before the occurrence of nucleophilic attack. A large number of systems has been studied in which preequilibrium complexation changes the reactivity of a substrate to nucleophilic attack.[18] Although complexation generally has been found to result in decreased reactivity of the substrate, a few cases have been noted in which increased reactivity occurs.[18,19] In agreement with the idea of preequilibrium complex formation are the curved buffer dilution plots obtained for the reaction of the arene oxide with the amines that exhibit the positive deviations in the Bronsted plot (Figure 3).[20] Saturation of substrate by buffer is typical of complexation effects. The observance of saturation in buffer dilution plots has also been taken as evidence of nucleophilic attack on an intimate ion pair; at low nucleophile concentrations, attack on the ion pair is rate limiting, with ion pair formation becoming rate limiting as the concentration of nucleophile is increased. Since the observed rate of attack of thiolate anion on phenanthrene 9,10-oxide is considerably faster (at high thiol concentration) than the saturation rate (apparent rate of ion pair formation) of Figure 3, one of two situations must pertain: (1) amines and thiols undergo SN2 addition, and saturation by amines is due to complex formation; or (2) the highly polarizable thiol anion undergoes an SN2 addition, while the less polarizable amine reacts with the intimate ion pair. Against the latter possibility is the observation that the saturation rate constant is amine dependent. The widely varying pK_a's and similar steric requirements of the four quinuclidine amines give rise to a satisfactory β_{nuc} value for the reaction of these amines with phenanthrene 9,10-oxide (Figure 7). That the value is markedly smaller (β_{nuc} = 0.1) than that obtained with primary and secondary amines (β_{nuc} = 0.4) suggests that two different mechanisms are involved, i.e., direct nucleophilic attack with primary and secondary amines and preequilibrium complexation, followed by nucleophilic attack with certain tertiary amines.

Polycyclic aromatic hydrocarbons, and presumably their arene oxides as well, are known to form complexes with purines and nucleic acids.[21] Phenanthrene 9,10-oxide and other K-region arene oxides bind covalently to DNA and RNA molecules,[4] with the purine bases (particularly guanine) exhibiting the greatest reactivity toward the arene oxides.[22] The results of the present study would suggest that the reaction of phenanthrene 9,10-oxide with tertiary amines serves as a model for the enhancement in rate of alkylation brought about by preequilibrium complexation of nucleophile and arene oxide. Our attempts to investigate the reaction of nucleotides with phenanthrene 9,10-oxide were not successful because of the strong absorbance of the bases in the same spectral region where the arene oxide and its nucleophilic addition products have their absorption maxima. However, since the arene oxide has been found to undergo nucleophilic attack by amines of pK_a as low as 4.6, it would be very surprising if it did not react with nucleic acid bases, particularly if it were intercalated.

Reaction with Thiols. From a comparison of the second-order rate constants for amines and oxygen bases (Table I) and thiolate anions (Table II), it is evident that the latter exhibit considerably greater nucleophilic reactivity toward phenanthrene 9,10-oxide. As a result of this greater nucleophilic reactivity, the thiolate anions, unlike amines and oxygen bases, react with phenanthrene 1,2-oxide and phenanthrene 3,4-oxide; nucleophilic attack has become sufficiently rapid to compete effectively with the spontaneous aromatization reactions. The second-order rate constants for the reaction of thiolate anions with these non-K-region arene oxides are given in Table II. The data of Table II indicate that the K-region arene oxide of phenanthrene is only slightly more susceptible to nucleophilic attack than are phenanthrene 1,2- and 3,4-oxides. *Thus, the K-region oxide is not inherently a better alkylating agent. Rather it is the decreased rate of the ring-opening reaction when the oxide is at the K-region and the decreased rate of the NIH shift off the carbonium ion that allows the K-region oxide to exist in solution long enough to express its potential as an alkylating agent.* It is apparent from the previous paper that all of the K-region arene oxides that we have investigated are associated with a much slower rate of ring opening than are the non-K-region oxides.

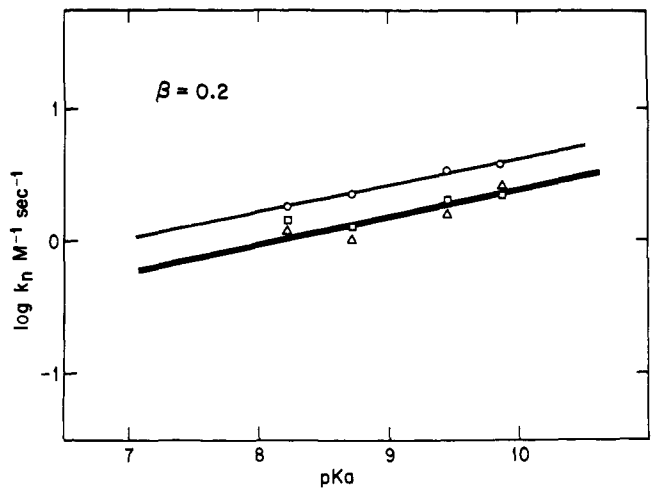


Figure 9. Bronsted plots for the reaction of thiolate anions with phenanthrene 9,10-oxide (○), phenanthrene 1,2-oxide (△), and phenanthrene 3,4-oxide (□) vs. the pK_a of the thiol.

It has recently been reported that benzene oxide undergoes nucleophilic attack with thiolate ions but not with primary and secondary amines or oxygen bases.[5] We have found that naphthalene 1,2-oxide shows similar behavior. As with the non-K-region arene oxides of phenanthrene, attack by amine and oxygen nucleophiles is too slow to compete with the aromatization of benzene or naphthalene 1,2-oxide, while thiolate anion attack is sufficiently rapid that it exceeds the aromatization reaction. The non-K-region arene oxides may be able to react with the very reactive tertiary amines (trimethylamine and quinuclidine amines), since these may be of sufficient reactivity to successfully compete with the aromatization reaction.

The Bronsted plots for the reaction of thiolate anions with the three phenanthrene oxides are given in Figure 9. The slopes of these plots are all about 0.2, indicating—assuming a Hammond-like reaction coordinate—a somewhat earlier transition state than is realized with attack by primary and secondary amines. The Bronsted β_{nuc} value for the attack of thiolate anions on benzene oxide was also found to be 0.2.[5] A decreased sensitivity of rate constants to pK_a for sulfur nucleophiles as compared with amines is also seen with *p*-nitrophenyl acetate where β_{nuc} for thiols is 0.4[23] as compared with β_{nuc} = 0.8 for amines.[24] Ethylene oxide, however, exhibits a slightly greater sensitivity to the basicity of thiols (β_{nuc} = 0.4)[25] than to amine basicity (β_{nuc} = 0.3). The reactivity of thiolate anions toward phenanthrene 9,10-oxide and ethylene oxide are correlated by eq 6.

$$\log k_{n(\text{phenanthrene 9,10-oxide})} = 0.35 \log k_{n(\text{ethylene oxide})} + 0.96 \quad (6)$$

At relatively high thiol concentrations (>~0.2 M), a second reaction following thiolate anion addition can be detected. This second reaction is likely attributable to aromatization of the addition product. The ease with which these α-thio alcohols aromatize became evident when attempts were made to acetylate the thiol addition products for the NMR studies. All other adducts examined were readily converted to acetylated derivatives.

Glutathione is known to react in vivo with arene oxides and thus functions as a natural detoxification agent for metabolically formed arene oxides.[26] In view of the products obtained from the reaction of thiolate anions with phenanthrene 9,10-oxide, the enzyme-catalyzed addition of glutathione more than likely takes place via trans 1,2 addition. It has been inferred that glutathione is unique in its ability to react with these intermediates.[27] From the Bronsted plots of Figure 9, and as noted previously from a like plot for the reaction of thiolate anions with benzene oxide,[5,28] it is apparent that glutathione is no more reactive a thiol than its pK_a would predict. Thus, any thiol, whether it be on a protein or a small molecule, is capable of reacting with a metabolically formed arene oxide. For low molecular weight thiols and nonessential proteins, this process represents detoxification. It is interesting to note that the enzyme-catalyzed reaction between arene oxides, simple epoxides, and glutathione appears to be highly specific for glutathione.[29] This apparent requirement for glutathione must reside in the specificity for this thiol as a cosubstrate for the enzyme and not in any intrinsic nucleophilicity of glutathione.

Product Studies. The chemical structure of the nucleophilic adducts were determined only with phenanthrene 9,10-oxide as substrate since this was the only arene oxide studied for which sufficient amounts of adducts could readily be obtained for analysis. In principal, the kinetics are consistent with any of three types of products: direct trans addition, cis addition, and remote addition in which the aromaticity of the biphenyl system is destroyed, or combinations thereof. Oxygen, nitrogen, and sulfur nucleophiles were examined (Table III). Nuclear magnetic resonance spectra were determined on crude reaction mixture extracts in order to identify the nature of the initial adducts present prior to decomposition of any labile species. In almost all cases, reactions proceeded to single products which had resulted from direct opening of the 9,10-oxirane ring. There were no instances in which detectable amounts of resonances due to vinyl hydrogens resulting from allylic attack through one of the benzene rings of the biphenyl system were observed. Only in the case of attack by azide was there a detectable amount (TLC and NMR) of fully aromatic product (9-azidophenanthrene) by the time all the starting phenanthrene 9,10-oxide had been consumed. The 9-azidophenanthrene is presumed to arise via partial dehydration of the initial adduct during the course of the reaction. Reaction conditions were such that 9-phenanthrol was not a significant product.

Chemical shifts for the ring hydrogens at the 9 and 10 positions and coupling constants ($^3J_{9,10}$) for the adducts and their acetylated derivatives as well as selected literature values are given in Table III. As established above, the adducts must consist only of trans isomers, cis isomers, or mixtures thereof. The adducts could only be mixtures if the cis and trans isomers have identical chemical shifts and coupling constants, a highly unlikely situation. Even more unlikely would be the situation in which the derived acetates of both isomers had identical chemical shifts and coupling constants. Since the initial adducts as well as the derived acetates gave no evidence for more than one product, it is necessary to conclude that these additions are stereospecific and that the adducts are probably trans.

For trans adducts, two extreme conformations are possible in which the hydrogens at the 9 and 10 positions occupy either pseudoaxial or pseudoequatorial positions. For a cis adduct, one of these hydrogens must occupy a pseudoaxial position and the other a pseudoequatorial position in either extreme conformer, a situation in which the coupling constant would not be expected to change on acetylation even if the most populated state changes. The methoxide adduct (Table III, entry 1) shows a large coupling (8.4 Hz) which is diminished on acetylation (4.6 Hz). Hydrogen bonding between OH and OCH_3 forces the hydrogens at the 9 and 10 positions into axial environments, while acetylation eliminates hydrogen bonding and increases steric bulk. Once acetylated, the molecule prefers the conformation with pseudoaxial substituents and a coupling constant of 4.6 Hz. Comparison with related cis and trans isomers of known configuration (Table III, entries 2 and 3) confirms that the methoxide adduct is indeed trans.

Adducts resulting from the addition of 2-mercaptoethanol and thiolacetic acid to phenanthrene 9,10-oxide (entries 4 and 5, Table III) were found to have unusually small coupling constants ($^3J_{9,10}$ = 3.9 and 3.5, respectively) for trans isomers. On attempted acetylation, the mercaptoethanol adduct aromatized. Acetylation of the thiolacetic acid adduct decreased the coupling constant to 2.7 Hz as expected for a trans isomer as described above. Thiol adducts of benzene oxide had previously been noted to have unusually small coupling constants (entry 6 and ref 4) for trans isomers. Whether the small coupling constants are due to conformational effects, to electronic effects of the sulfur substituent,[30] or to a combination of both is presently unknown.

The relative stereochemistry of the adducts obtained with several nitrogen nucleophiles (entries 7–12) was also investigated. In contrast to a previous study of nucleophilic reactions of benzene oxide and naphthalene 1,2-oxide[5a] which were unreactive toward amine nucleophiles, the 9,10-oxide (K-region) of phenanthrene was found to react rather readily with amines and azide. In each case, single isomers were observed with values of the $^3J_{9,10}$ coupling constant ranging from 5.5 to 9.4 Hz, which is consistent with trans stereochemistry. The coupling constant for the dimethylamine adducts (entries 8 and 9) are the smallest and least convincing for trans isomers, but are consistent with the hydroxyl and amino substituents mainly in pseudoaxial environments. Steric hindrance may be an important factor in destabilizing intramolecular hydrogen bonding in these adducts which are tertiary amines. Acetylation of the dimethylamine, *tert*-butylamine, and azide adducts gave evidence for single isomers in which the coupling constants were decreased to 3–5 Hz, which is consistent with trans stereoisomers. In contrast, acetylation of the adducts from methyl- and *n*-butylamine produced what appeared to be mixtures of stereoisomers. Restricted rotation and magnetic anisotropy of the amide group (Table III, footnote *c*) were responsible for the apparent mixtures since spectra determined at higher temperatures showed partial collapse to the expected signals for single trans isomers.

In summary, only direct trans opening of the K-region arene oxide was detected. This stereochemical result is consistent equally with direct SN2 opening of the oxirane ring and with nucleophilic trapping of tight ion pairs. Reaction of the unsymmetrical 3-bromophenanthrene 9,10-oxide with dimethylamine produced unequal amounts (55 and 45%) of the two possible trans adducts. Previous studies on the nucleophilic reactions of naphthalene 1,2-oxide[5a] established that predominant attack occurred at C-2, the site of the most stable carbonium ion. These observations do not allow an unequivocal selection between SN2 and tight ion pair trapping mechanisms, although the naphthalene 1,2-oxide results are suggestive of an ion pair mechanism. At present, the only nucleophilic reaction on an arene oxide in which a definite choice between the two mechanisms may be made is the reaction of ethyl mer-

Table IV. Relative Rates of Spontaneous Ring Opening (A) and Thiolate Anion Nucleophilic Attack (B)

Compd	A[a]	B[b]	B/A = nucleophilic susceptibility index
Ethylene oxide	1[c]	1[e]	1
Benzene oxide	2 000[d]	4[f]	0.002
Naphthalene 1,2-oxide	5 000[d]	41	0.008
Phenanthrene 1,2-oxide	50 000[d]	39	0.0008
Phenanthrene 3,4-oxide	88 000[d]	50	0.0006
Phenanthrene 9,10-oxide	300[d]	82	0.3

[a] Ethylene oxide measured at 25 °C, all others at 30 °C. [b] Attack on ethylene oxide measured at 20 °C, others at 30 °C. [c] Reference 12. [d] Reference 6. [e] Reference 25. [f] Reference 5.

captan with 4-chlorobenzene oxide,[31] where sulfur attacks at C-1. The reaction must be direct SN2 opening since 4-chlorophenol is the only product obtained upon isomerization of 4-chlorobenzene oxide. It is probable that other arene oxides with electron-withdrawing substituents will undergo rapid SN2 reactions with nucleophiles. The adduct from 4-chlorobenzene oxide and ethyl mercaptan undergoes an unusual migration via a sulfonium ion to produce 3-chlorophenyl ethyl thioether on dehydration.[31]

Relative Reactivities of an Aliphatic Epoxide and Arene Oxides toward Nucleophilic Attack. The relative rates of reaction in water, under conditions where opening of the epoxide ring is rate limiting, are given in column A of Table IV for ethylene oxide, four non-K-region arene oxides (benzene oxide, naphthalene 1,2-oxide, phenanthrene 1,2-oxide, and phenanthrene 3,4-oxide), and a K-region arene oxide (phenanthrene 9,10-oxide). Of the six compounds, only ethylene oxide and phenanthrene 9,10-oxide undergo nucleophilic attack by amines and hydroxide ion. From the relative rates of ring opening, it is apparent that in the case of these two compounds the nucleophiles have the best chance of competing with the solvolysis reaction. For the other four compounds, the ring opening reaction (which is followed by a rapid NIH shift and aromatization) is faster than the rate of nucleophilic attack by amines and hydroxide ion.

The greater nucleophilic activity of thiolate anions as compared with amines allows them to effectively compete with the solvolysis reaction. The relative second-order rate constants for attack by 2-mercaptoethanol are given in column B. Of the six compounds listed, the K-region arene oxide is the most susceptible to attack by thiolate anion. Since both susceptibility to nucleophilic attack and the length of time it exists in solution (i.e., is free from competing reactions) are important in determining whether an arene oxide will be subject to nucleophilic addition reactions, we have defined B/A as an index of nucleophilic susceptibility. The index for each of the six compounds is given in Table IV. The K-region arene oxide is clearly quite similar in its reactivity as an alkylating agent to a normal aliphatic epoxide and quite different from the non-K-region arene oxides.

The effect of intercalation into DNA on the reactivity of arene oxides has not been evaluated, although the greatly enhanced reactivity of phenanthrene 9,10-oxide upon complexing with quinuclidine amines may well be suggestive of what will be found. K-region arene oxides are relatively inactive as carcinogens in vitro, but have been found to be potent transforming agents for mammalian cells in culture.[3b] Comprehensive comparisons of the biological activity of K-region vs. non-K-region arene oxides are not yet available. A recent study on the mutagenicity of benzo[*a*]pyrene 7,8- and 9,10-oxides (non-K-region) vs. benzo[*a*]pyrene 4,5-oxide (K-region) has established that the K-region arene oxide is a far more potent mutagen toward bacterial cells and mammalian cells in culture.[32] Parallel studies on the carcinogenicity of these arene oxides in vivo have indicated that benzo[*a*]pyrene 7,8-oxide is the only potent carcinogen.[33] The carcinogenicity of this arene oxide may be the result of conversion into a diol epoxide[34] which acts as the ultimate carcinogen.

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