California Chapter of the ARCS Foundation for a scholarship.

References and Notes

(1) Paper 39 in the Studies on Pituitary Lactogenic Hormone series.
(2) C. H. Li, J. S. Dixon, T. B. Lo, K. D. Schmidt, and Y. A. Pankov, *Arch. Biochem. Biophys.*, **141**, 705–737 (1970).
(3) A. Trenkle, C. H. Li, and N. R. Moudgal, *Arch. Biochem. Biophys.*, **100**, 255–259 (1963).
(4) W. C. Clarke and C. H. Li, *Arch. Biochem. Biophys.*, **161**, 313–318 (1974).
(5) J. Solis-Wallckermann, Ph.D. Thesis, University of California, San Francisco, 1972.
(6) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149–2154 (1963).
(7) B. Iselin, *Helv. Chim. Acta*, **44**, 61–78 (1961).
(8) A. Loffet, *Int. J. Protein Res.*, **3**, 297–299 (1971).
(9) D. Yamashiro and C. H. Li, *J. Am. Chem. Soc.*, **95**, 1310–1315 (1973).
(10) T. Wieland, C. Birr, and F. Flor, *Angew. Chem.*, **83**, 333–334 (1971).
(11) H. Hagenmaier and H. Frank, *Hoppe-Seyler's Z. Physiol. Chem.*, **353**, 1973–1976 (1972).
(12) Abbreviations: TFA, trifluoroacetic acid; CM-cellulose, carboxymethylcellulose; TLC, thin-layer chromatography; BAW, 1-butanol–acetic acid–water, 4:1:1 (v/v); BPAW, 1-butanol–pyridine–acetic acid–water, 15:10:3:12 (v/v); DCC, *N,N'*-dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; Hsl, homoserine lactone; Hse, homoserine.
(13) In earlier attempts to synthesize prolactin-(37–55), less satisfactory results were obtained when the coupling reactions were performed by the standard in situ formation of the anhydrides by DCC [J. Rebek and D. Feitler, *J. Am. Chem. Soc.*, **96**, 1606–1607 (1974)], particularly when the starting Boc-Leu resin was prepared by the triethylamine method (see ref 6).
(14) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164–2167 (1967); J. Lenard and A. B. Robinson, *J. Am. Chem. Soc.*, **89**, 181–182 (1967); R. H. Mazur and G. Plum, *Experientia*, **24**, 661 (1968).
(15) E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751–755 (1956).
(16) D. Yamashiro, *Nature (London)*, **201**, 76 (1964).
(17) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190–1206 (1958).
(18) H. G. Gundlach, S. Moore, and W. H. Stein, *J. Biol. Chem.*, **234**, 1761–1764 (1959).
(19) Trace amounts of the *S-tert*-butylsulfonium ion form of human calcitonin M resulting from the final deprotection of the protected peptide synthesized in solution by fragment condensation have been reported but not characterized: B. Riniker, M. Brugger, B. Kamber, W. Rittel, and P. Sieber in "Progress in Peptide Research, Vol. II, Proceedings of the Second American Peptide Symposium", S. Lande, Ed., Gordon and Breach, New York, N.Y., 1972, p 116.
(20) D. Yamashiro, M. Rigbi, T. A. Bewley, and C. H. Li, *Int. J. Pept. Protein Res.*, **7**, 389–393 (1975).
(21) B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 6165–6170 (1972).
(22) B. F. Gisin, *Anal. Chim. Acta*, **58**, 248–249 (1972).
(23) D. Yamashiro and C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 4945–4949 (1974).
(24) D. Yamashiro and C. H. Li, *J. Org. Chem.*, **38**, 591–592 (1973).
(25) D. Yamashiro, J. Blake, and C. H. Li, *J. Am. Chem. Soc.*, **94**, 2855–2859 (1972).
(26) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688–5691 (1959).
(27) B. Hemmasi and E. Bayer, *Hoppe-Seyler's Z. Physiol. Chem.*, **355**, 481–489 (1974).
(28) T. Baba, H. Sugiyama, and S. Seto, *Chem. Pharm. Bull.*, **21**, 207–209 (1973).
(29) P. J. Vithayathil and F. M. Richards, *J. Biol. Chem.*, **235**, 2343–2351 (1960).
(30) M. R. Sairam, W. C. Clarke, D. Chung, J. Porath, and C. H. Li, *Biochem. Biophys. Res. Commun.*, **61**, 355–359 (1974).
(31) E. Wasserman and L. Levine, *J. Immunol.*, **87**, 290–295 (1961).

Polycyclic K-Region Arene Oxides. Products and Kinetics of Solvolysis

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Abstract: K-Region oxides derived from four polycyclic aromatic hydrocarbons (phenanthrene 9,10-oxide (**1**), benz[*a*]anthracene 5,6-oxide (**2**), dibenz[*a,h*]anthracene 5,6-oxide (**3**), and 3-methylcholanthrene 11,12-oxide (**4**) solvolyze in 1:1 dioxane–water, 0.10 M in KCl (cf. ref 14 for oxide **1**). At pH's 3–6 the oxides **1–4** quantitatively produced 3:1 mixtures of K-region phenols and dihydrodiols, respectively. Oxide **4** gave the same ratio up to pH 10 and a 1:1 ratio at pH 12. Liquid chromatography of the dihydrodiols showed that the mole percent cis isomer varied with the oxide: **1**, 20% *cis-*; **2**, 31% *cis-*; **3**, 32% *cis-*; **4**, 75% *cis-*. Oxide **4** produced equal cis/trans dihydrodiol ratios at pH's 3, 4, and 5. Diazomethane methylation of the product phenols, followed by comparison of the NMR or the gas chromatogram of the mixtures with those of the authentic ethers, showed the relative amounts of the positional isomers to be: **2**, 60:40, 5:6; **3**, 85:15, 5.6; **4**, >98:2, 11:12. Uv spectral analysis of oxide solvolyses carried out in a pH stat at 36.8 °C indicated first-order disappearance of oxides **1–4** and simultaneous appearance of phenols and dihydrodiols at the same rate. The first-order rate constant for oxides **1–3** can be expressed as $k_{obsd} = k_H a_H$ and that of oxide **4** is $k_{obsd} = k_H a_H + k_0$. Values for k_H (M^{-1} sec^{-1}) were: **1**, 8.09; **2**, 14.8; **3**, 19.3; **4**, 990. The k_0 value for oxide **4** was 3.07×10^{-5} sec^{-1}. These results can be rationalized by a scheme which includes rate-limiting ring opening of the protonated or unprotonated oxide ring, followed by competitive water attack on or NIH shift of the intermediate benzylic carbocation. Rate-limiting formation of the 11*H*-11-hydroxy-3-methylcholanthren-12-yl cation may be accelerated by inductive stabilization of the delocalized incipient carbocation.

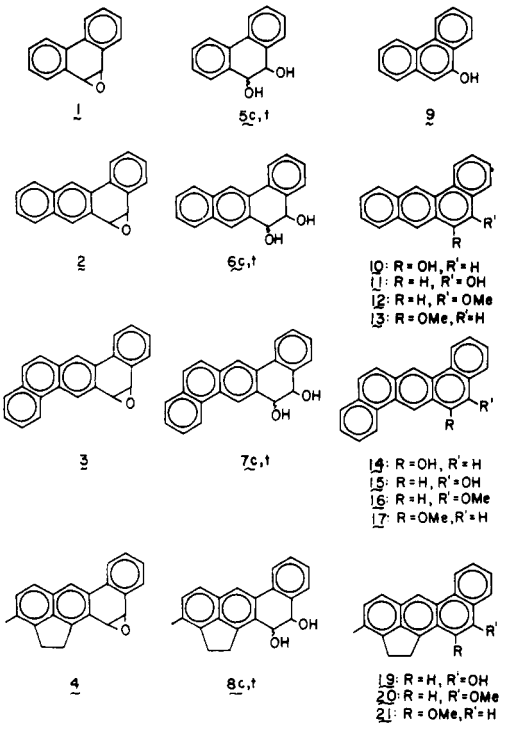
Introduction

Polycyclic arenes are an important class of carcinogenic compounds. The work of the Millers[1] and others has demonstrated that most chemical carcinogens, including polycyclic arenes, must be metabolically activated before they can be covalently bound to cellular macromolecules. Such binding is considered necessary for cell transformation.[2] DNA may be the target for binding, but this has not yet been proven. Evidence has accumulated that arene oxides are one type of reactive metabolite of polycyclic arenes.[3] The conversion of arenes to arene oxides is carried out by membrane-bound cytochrome P-450 monooxygenases.[4]

Several K-region[5] arene oxides, including phenanthrene 9,10-oxide[6] (**1**), benz[*a*]anthracene 5,6-oxide[6] (**2**), and dibenz[*a,h*]anthracene 5,6-oxide[7] (**3**), have been detected as metabolites when the parent arenes were added to fortified rat liver microsomes. The biological effects of these and other arene oxides have been studied in our laboratory. It was demonstrated that **3** and 3-methylcholanthrene 11,12-oxide (**4**) were more active than the parent arenes or other

K-region derivatives in producing oncogenic transformation[8] and mutations[9] in mammalian cell cultures. Arene oxides have also been shown to bind to DNA in solution[10] and in cell cultures.[2,10]

Any attempt to understand these biological phenomena must be based on an understanding of the controlling factors in arene oxide reactivity, especially the nonenzymatic phenol production. Bruice and others[11-14] have explored the chemistry of several arene oxides, both K-region and non-K-region. However, those studies were restricted to oxides of noncarcinogenic arenes. We have studied the products and kinetics of the phenol-producing reactions of four polycyclic arene oxides, two of which are derived from carcinogens (**3** and **4**) and two, from noncarcinogens (**1** and **2**).



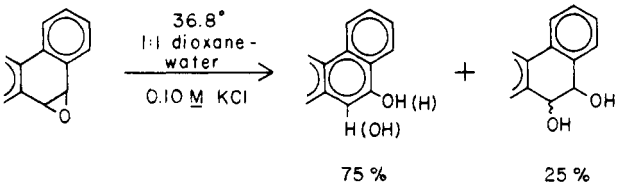
Results

Products of Solvolysis. K-Region oxides **1–4** not only rearrange in acidic dioxane–water to K-region phenols but also add a mole of water to give stable K-region dihydrodiols in substantial amounts (20–30% yield) (Tables I and II). The identities and relative amounts of these two sets of products were determined both by physical separation with spectral identification and by spectrophotometric studies of the final reaction mixtures.

Table II. Percent Yield of Phenols as Determined by Computer Analysis of the Uv Spectrum of the Final Reaction Mixtures

Starting material	pH of reaction	Calcd mol % phenol
3-Methylcholanthrene 11,12-oxide (**4**)	4.70	71.0
	5.00	72.4
	6.10	72.8
	6.77	74.5
	8.00	80.0
	9.00	76.0
	10.00	80.0
	11.00	75.1
	12.00	50.0
Dibenz[*a*,*h*]anthracene 5,6-oxide (**3**)	3.70	76.6
	3.98	80.7
	4.20	73.0
	4.50	75.0
	5.40	76.9
Benz[*a*]anthracene 5,6-oxide (**2**)	3.60	67.0

The reaction products from acidic solvolysis of arene oxides **3** and **4** in 1:1 dioxane–water, 0.10 M in potassium



chloride, were distributed between layers of hexane and 1 N aqueous potassium hydroxide, causing the phenols (as phenoxides) to be extracted into the aqueous layer while the dihydrodiols remained in the hexane. Analysis of the uv spectra of both phases using ϵ values calculated from the spectra of authentic compounds[15,16] showed about a 25% yield of dihydrodiols from the reaction of each oxide (Figures 1–3). The phenol and dihydrodiols from reaction of oxide **1** were separated by TLC, and the extracted spots were assayed spectrophotometrically.

Additional evidence for the presence of stable K-region dihydrodiols in the solvolysis products was found in the stable uv spectra of the untreated final reaction mixtures of oxides **2–4** (Table II). They showed λ_{max}'s due both to K-region dihydrodiols and phenols (Figures 1–3). The ratios of phenols to dihydrodiols were calculated from these spectra knowing the λ_{max}'s and ϵ values of the dihydrodiols and phenols. (Cis and trans dihydrodiols have very similar uv spectra; the mixtures of the K-region phenols prepared by

Table I. Products of Reaction of K-Region Arene Oxides in 1:1 (v/v) Dioxane–Water, 0.10 M in KCl

Oxide	% yield of dihydrodiols produced in acidic reaction	Retention time, min[d] Cis	Retention time, min[d] Trans	% cis dihydrodiols of total diols (reaction pH)	Ratio of isomeric aryl methyl ethers from methylation of phenols (OMe position) from acid-catalyzed rearrangement
1	19[a]	35	26	20[d] (2.50)	
2	33[b]	21	12	31[d] (2.67)	60:40[f] (5, 6)
3	22[b,c]	33	22	32[d] (2.85)	85:15[g] (5, 6)
4	26[b,c]	26	34	75[d,e] (2.85, 3.96, 4.90)	>98:2[f] (11, 12)
				71[h] (3.0)	
				62[h] (9.00)	
				55[e,i] (11.00)	

[a] By TLC separation of phenols and diols produced at pH 1.0, followed by quantitative uv of extracted spots. [b] By computer analysis of uv spectra of final phenol-diol mixtures at pH's 3–6. [c] By extraction of phenols from dihydrodiols with aqueous KOH, then uv analysis of the separated layers. [d] By HPLC on Dupont No. 830, 25 × 0.65 cm Zorbax column, eluted with 6% v/v (methylene chloride:2-propanol:acetic acid, 1000:20:0.1) in cyclohexane 0.2 ml/min at 25 °C. [e] By separation of boric acid soaked silica gel sheets and quantitative uv spectra of the extracted spots. [f] By comparison of the 90-MHz NMR of the mixture with those of the separate ethers. [g] By comparison of gas chromatography retention times with those of the authentic ethers. [h] By HPLC as above with reaction at 37°. [i] Produced from reaction at 47°.

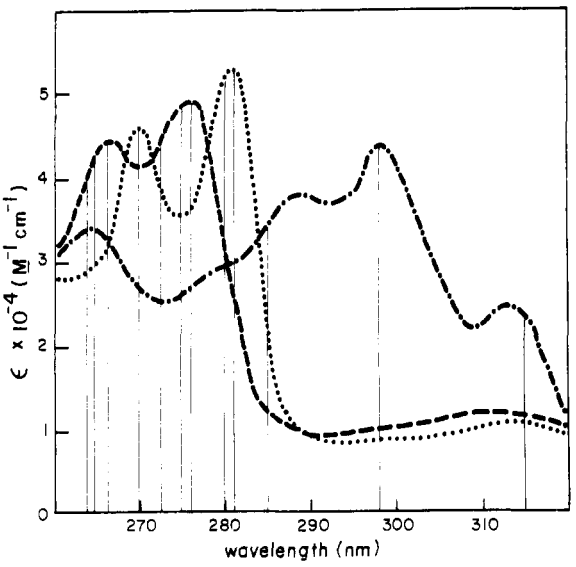


Figure 1. Uv spectra in 1:1 dioxane–water, 0.10 M in KCl, of various K-region derivatives of benz[*a*]anthracene: (·····) 5,6-oxide (**2**); (- - - -) -5,6-dihydrodiol (**6**), (-·-·-), 5- + 6-hydroxy- (**10** + **11**). Regression analysis wavelengths are indicated by the vertical lines.

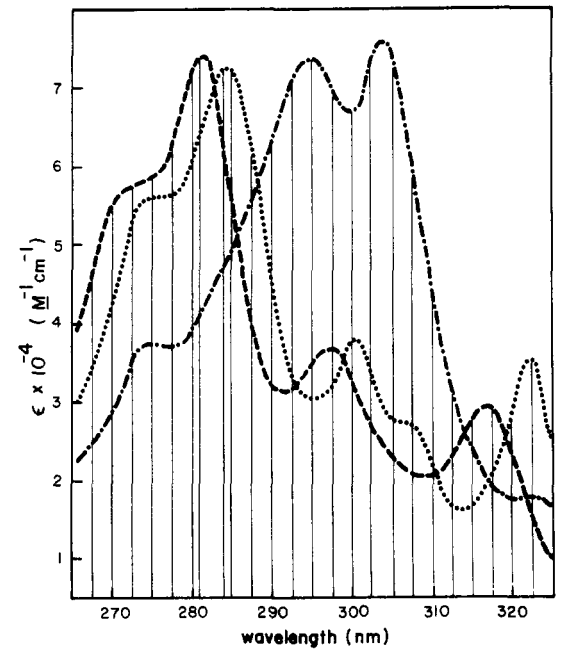


Figure 2. Uv spectra in 1:1 dioxane–water, 0.10 M in KCl, of various K-region derivatives of dibenz[*a,h*]anthracene: (·····) 5,6-oxide (**3**), (- - - -) -5,6-dihydrodiol (**7**), (-·-·-) 5- + 6-hydroxy- (**14** + **15**). Regression analysis wavelengths are indicated by the vertical lines.

literature methods were similar in isomeric composition to those obtained by arene oxide solvolysis.[17]) Although a simple two-wavelength analytical method might have sufficed, we developed and used a computerized multiwavelength linear regression analysis[18] (see Experimental Section) suitable for analyzing binary or tertiary mixtures of K-region oxides and/or phenols and/or dihydrodiols. The results shown in Table II are in agreement with those obtained with the extraction method. Control experiments in which dihydrodiols **5c, 5t, 7t, 8c,** and **8t** were held for at least 15 oxide half-lives showed no formation of phenols or cis–trans dihydrodiol isomers.

Spontaneous oxide ring opening was observed for 3-methylcholanthrene oxide (**4**) at pH 8–12; the other oxides were not detectably reactive at these pH's. From pH 8 to

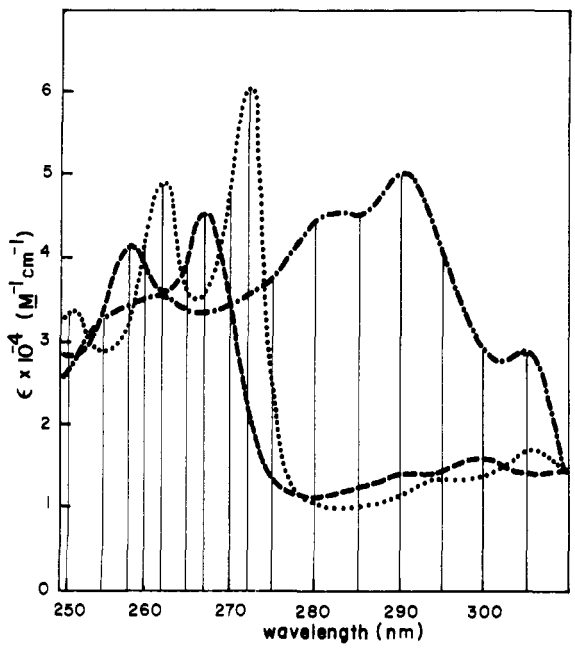


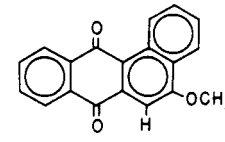
Figure 3. Uv spectra in 1:1 dioxane–water, 0.10 M in KCl, of various K-region derivatives of 3-methylcholanthrene: (·····) 11,12-oxide (**4**), (- - - -) -11,12-dihydrodiol (**8**), (-·-·-) 11-hydroxy- (**19**). Regression analysis wavelengths are indicated by the vertical lines.

11, oxide **4** gave 20–25% yields of K-region dihydrodiols; at pH 12.0 the dihydrodiol yield increased to 50%, as determined by spectrophotometry of the final mixture.

Facile separation of cis and trans dihydrodiols **5–8** by high-pressure liquid chromatography allowed determination of the cis/trans ratios of the product dihydrodiols (Table I). This ratio changed dramatically from compound to compound, with predominant cis dihydrodiol (75% cis) formed from oxide **4,** to almost exclusive trans dihydrodiol formed from oxide **1** (19% cis). The percent cis dihydrodiol from oxide **4** was insensitive to pH changes in the acid range (72.8, 72.9, and 73.2% cis at pH 2.84, 3.96, and 4.90, respectively) but decreased to 62% at pH 9 and 55% at pH 11.

The product K-region phenols also were produced in isomeric mixtures (Table I). The authentic ethers **16** and **17**[19] of dibenz[*a,h*]anthracene were separated by gas chromatography, and the ethers produced by diazomethane methylation of phenols **15** and **14** obtained from the acid-catalyzed rearrangement of oxide **3** were similarly separated. Peak integration showed that **16** predominated in the ratio 85:15.

Separation of ethers **12** and **13** from methylation of benz[*a*]anthracene phenols **11** and **10** was not achieved with either gas- or high-pressure liquid chromatography. However, 90-MHz ^{1}H NMR of the mixture of ethers showed two methoxyl signals at δ 4.12 and 4.15, the upfield one predominating in the ratio of 3:2.[17] The assignment of the upfield signal to **12** was strengthened by preparation of each ether separately. The **12** + **13** mixture was first oxidized to a mixture of methoxyquinones **22** + **23,** which were separated by alumina column chromatography. Hydride reduc-



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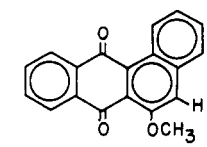

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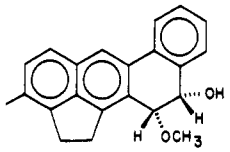
tion of the quinones gave back the isomeric methoxybenz[*a*]anthracenes. Especially revealing were the NMR chemical shift differences of the methoxy singlets (δ 4.12 for **12** and δ 4.15 for **13**), the K-region aryl proton singlets (δ 7.02 for **12** and δ 6.88 for **13**), and the H-7 peri-position singlets (δ 8.21 for **12** and δ 8.85 for **13**).

Similarly, the phenols from acid-catalyzed solvolysis of 3-methylcholanthrene oxide (**4**) were methylated. However, 90-MHz ^{1}H NMR of the methyl ether(s) showed only one methoxyl singlet and one K-region aryl proton singlet. This was assigned the structure of 11-methoxy-3-methylcholanthrene (**20**) by comparing its ^{1}H NMR spectrum with that of 12-methoxy-3-methylcholanthrene (**21**). Ether **21** was synthesized by pyridine-tosyl chloride dehydration of alcohol **24**, produced along with **25** in the acidic methanolysis of oxide **4** (see Experimental Section). Comparison of the 90-



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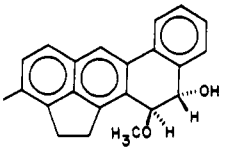

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MHz nmr spectra of the two methoxy compounds was instructive. 11-Methoxy-3-methylcholanthrene (**20**) has a four-proton doublet at δ 3.38 and 3.55 due to the hydrogens on carbons 1 and 2. The parent hydrocarbon has a very similarly shaped doublet at δ 3.35 and 3.62.[20] The isomeric methoxy compound **21** has one-half of the alkyl bridge doublet at the same chemical shift, δ 3.38; the other half is downfield at δ 3.98. This is due to deshielding by the neighboring 12-methoxy group. In addition, H-11 in **21** is upfield from H-12 in **20**; this parallels the chemical shift differences of the K-region protons in the parent hydrocarbon.[20]

Kinetics of Solvolysis. The reaction kinetics of oxides **3** and **4** were studied at a constant pH in a Radiometer pH stat at 36.8°. The product phenols and dihydrodiols appeared in parallel first-order processes with both rate constants equal to the first-order rate constant (k_{obsd}) for oxide disappearance. Changes in concentration of each of these species with time were followed in the reactions of oxides **3** and **4**. This was accomplished by computer analysis of the uv scan (250–340 nm) of each quenched aliquot. Plots of ln [|(mole percent)$_\infty$ − (mole percent)|] vs. time were linear through 90% reaction in all cases.

Such a complete spectral analysis was not possible in the case of phenanthrene 9,10-oxide (**1**) because the ϵ values of phenol **9** are much larger than the dihydrodiol ϵ values. However, measurement of the change in aliquot absorbance at the phenol λ_{max} (248 nm) and plots of ln ($A_\infty - A$) vs. time of quenching did give good first-order rate constants. The reaction of benz[*a*]anthracene 5,6-oxide (**2**) in the same solvent gave similar results by the latter method.

Oxides **1–4** reacted at different rates at a given pH, but responded to changes in pH identically, the k_{obsd} values being directly proportional to the hydrogen ion activity determined by a glass electrode (Figure 4). The slopes of the log k_{obsd} vs. pH plots were −1.0 for each oxide; the antilogs of the intercepts, k_H, varied by two orders of magnitude from oxide **1** to **4** (Table III).

3-Methylcholanthrene 11,12-oxide (**4**) was unique among the arene oxides studied in that it displayed solvolytic reactivity at pH's 8–12 (Figure 4). In spite of the changes in product distribution with change in pH (see above), there was no change in the observed first-order rate constant, $k_0 = 3.07 \pm 0.22 \times 10^{-5}$ sec^{-1}, with pH in the pH range 9–12. As in the acid pH region, the change in

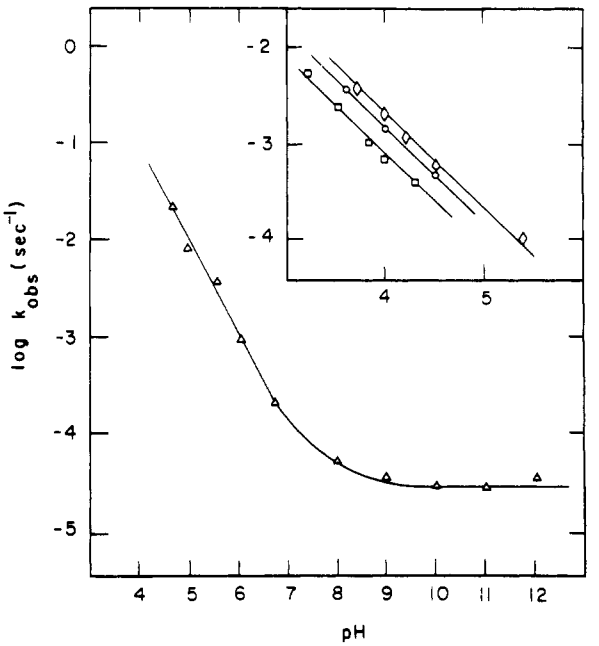

Figure 4. Plots of log k_{obsd} (s^{-1}) vs. pH for solvolysis of various arene oxides in 1:1 dioxane-water, 0.10 M in KCl, at 36.8 °C: □, **1**; ○, **2**; ◇, **3**; △, **4**.

Table III. Kinetic Results for the Reactions of Various Arene Oxides in 1:1 Dioxane-Water (KCl = 0.10 M) at 36.8°

Compound	k_H, M^{-1} sec^{-1}, $\pm 1\sigma$	k_H(rel)
Phenanthrene 9,10-oxide (**1**)	8.09 ± 0.61	1.00
Benz[*a*]anthracene 5,6-oxide (**2**)	14.8 ± 0.42	1.83
Dibenz[*a*,*h*]anthracene 5,6-oxide (**3**)	19.3 ± 2.2	2.39
3-methylcholanthrene 11,12-oxide (**4**)	990 ± 120	120

concentration of each species (oxide, phenol, and dihydrodiol) was monitored by uv scans of quenched aliquots; the three compounds showed equal first-order concentration changes.

Discussion

The present results agree in part with a mechanistic scheme proposed by Kasperek et al.[11,12] and Bruice et al.[14] for the solvolysis of various oxides of benzene, naphthalene, and phenanthrene. They found that the rate-limiting step of both acid- and non-acid-catalyzed oxide solvolysis was carbon–oxygen bond breakage with formation of a carbocation and a vicinal hydroxyl or alkoxide.[12] Our studies on oxides **1–4** provide some insight into the rate-limiting formation of the intermediate carbocations and their subsequent reactions. Our results also complement and extend the qualitative reactivity studies of Swaisland et al. on oxides **1–4**.[21]

In the pH range 3–6, the oxides **1–4** undergo typical acid-catalyzed reaction with $k_{obsd} = k_H a_H$ (Figure 4). This can be rationalized by a kinetic scheme which includes a proton-transfer equilibrium between solvent and the arene oxide prior to the rate-limiting step[11,12,22] (Figure 5). Thus, the oxide **a** is in equilibrium with the protonated oxide **b**[23] (equilibrium constant $K_b = k_1(H^+)/k_{-1}$). Ion **b** then opens (with rate constant k_2) to the benzylic cation **d**. If the proton transfers[24] and the subsequent reactions of **d** are much faster than its formation, then the steady-state approximation can be applied to the concentrations of **b** and **d**.

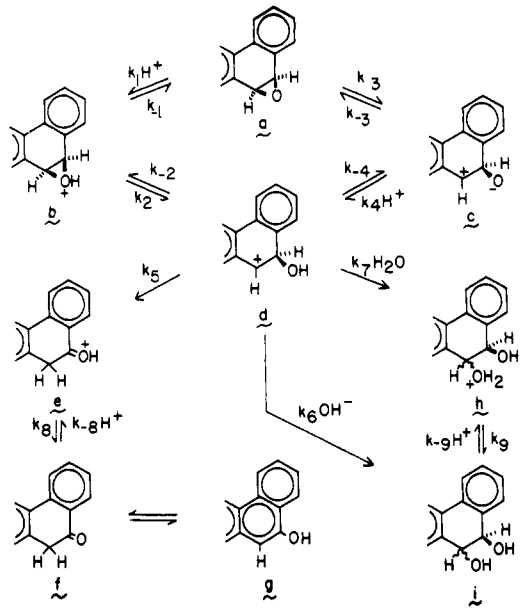

Figure 5. A mechanistic scheme that leads both to K-region phenols and K-region dihydrodiols from K-region oxide solvolysis. Only one of the two possible directions of ring opening is illustrated.

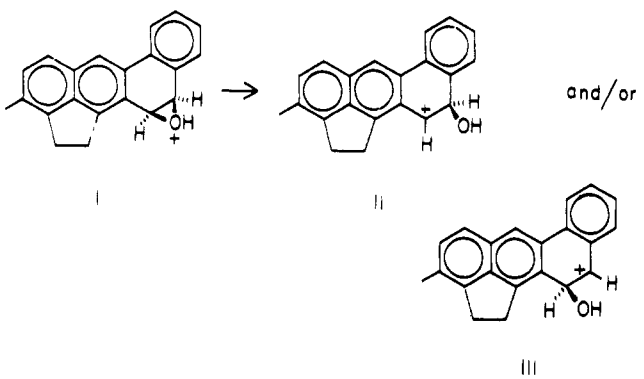

Figure 6. Two possible directions for the ring opening of protonated 3-methylcholanthrene oxide.

Solution of the resulting differential equations gives another expression for k_{obsd} i.e.,

$$k_{obsd} = K_b k_2 a_H$$

The rate-limiting step in the acid-catalyzed solvolysis of an asymmetric arene oxide can produce two isomeric benzylic cations. For example, in Figure 6, I can give either II or III. Since these ions subsequently isomerize to the protonated phenols (**19** and **18**, respectively), a difference in the activation energies of the two alternative ring-opening processes may account for the predominance of one phenol isomer over the other.[25] NMR analysis of the methylated phenols produced an estimate for this ratio of greater than 98/2.

Since C–O bond scission in I was nearly 70 times faster than the similar process in the nonalkylated benz[*a*]anthracene oxide solvolysis (assuming equal pK_b's), there must be a strong stabilizing influence on the transition state leading to II, compared to that leading to III or the similar benz[*a*]anthracenium ions.

One may attribute this strong effect to inductive stabilization of the transition state leading to II by alkyl groups attached to carbon atoms carrying partial positive charge. These atoms may be identified by the nonbonding MO

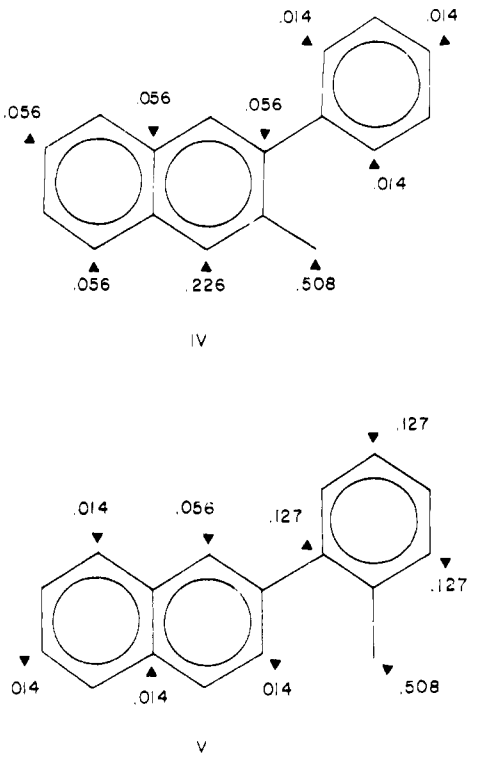

Figure 7. Hückel MO approximation of positive charge density in the two parent isomeric arylmethyl cations generated by scission of either the C_6–O bond of protonated benz[*a*]anthracene oxide (**IV**) or C_5–O bond (**V**). Triangles identify the charge-carrying atoms, which are labeled with the calculated fraction of charge residing at the carbon.

(NBMO) method of Longuet-Higgins,[26] which approximates the charge densities developing in the two isomeric parent ions, 3-phenyl-2-naphthylmethyl cation (IV) and 2-(2′-naphthyl)benzyl cation (V) (Figure 7). Alkyl substitution on carbons 1 and 8 in IV, where the positive charge density is especially large, would lead to stronger inductive stabilization of the transition state leading to that ion than would substitution on the same carbons in cation V, since these carbons are at nodes in the NBMO of V and carry no charge.

In addition to acid-catalyzed ring opening, 3-methylcholanthrene 11,12-oxide (**4**) undergoes non-acid-catalyzed solvolysis (Figure 4) similar to benzene oxide, naphthalene oxide, and the oxides of phenanthrene.[11,12,14] The rate-limiting step of this spontaneous process is probably the formation of zwitterion **c** from the oxide **a**[11] (Figure 5). While direct formation of products is possible from **c**, fast protonation of alkoxide oxygen to give cation **d** is more probable,[22] followed by fast product-forming reactions.

The product-forming steps that follow formation of the intermediate benzylic cation give rise both to K-region phenols and K-region dihydrodiols. The phenols probably form via 1,2-hydride shifts (NIH shifts[27]) to give the protonated keto form, **e**, of the phenol, **g** (Figure 5). The dihydrodiols, **i**, which arise from attack of water or hydroxide ion on the intermediate ion, **d**, have not previously been found as products of acid-catalyzed arene oxide solvolysis. Their formation is significant both chemically and biologically.

Attack of water on the intermediate cations from ring opening of each oxide produces both cis and trans dihydrodiols, with the cis/trans ratio being proportional to k_H. Similar phenomena have been observed recently by Battistini et al.[28] in the hydrolysis of a series of aryl-substituted oxiranes. Figure 8 illustrates possible transition states of the two directions of water attack on a benzanthracenium ion;

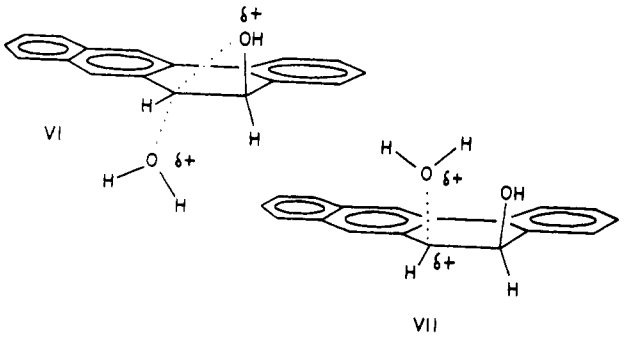

Figure 8. Possible transition states in formation of trans and cis dihydrodiols (VI and VII, respectively) from attack of water on a 5*H*-5-hydroxybenz[*a*]anthracen-6-yl cation.

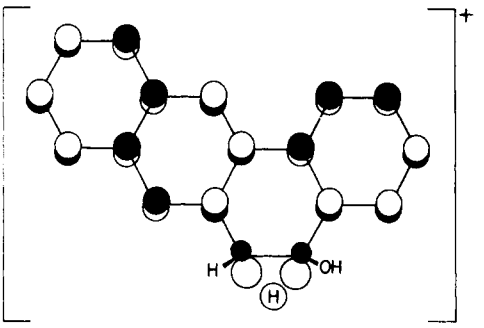

Figure 9. Transition state in the NIH rearrangement pathway of the 5*H*-5-hydroxybenz[*a*]anthracen-6-yl cation. Basis orbitals are shaded to represent HOMO of benz[*a*]anthracene[31] with a 1,6 (or 1,18) suprafacial hydrogen shift in progress.

note especially the difference in charge density at the reacting α carbon in the two transition states.

Dihydrodiol formation at high pH may result in part from attack of hydroxide ion on intermediate ion **d** (Figure 5). Here, the excess electron density on the hydroxide oxygen can transfer to the reacting α carbon in the transition state, which reduces the difference in positive charge density at the reacting α carbon between cis and trans attack. This effect may be seen in the increased yields of trans dihydrodiol from spontaneous solvolysis of oxide **4** at high pH's.[29]

Aryl-substituted oxiranes that are not arene oxides solvolyze to give high yields of dihydrodiols,[28] whereas arene oxides **1–4** give large amounts of rearrangement products (phenols) in addition to hydrolysis. A difference between these two types of oxides is that nonarene oxides contain an oxirane ring bonded to separate (i.e., nonconjugated) aryl groups, while arene oxides contain an oxirane whose carbons are bonded to one conjugated system. This structural feature may favor an NIH shift[27] by the intermediate cation, since the shift may occur by a symmetry-allowed process[30] whose transition state is stabilized by Hückel-like resonance (Figure 9). This stabilization is not available to ions arising from noncyclic oxides.

Of particular relevance to chemical carcinogenesis is our finding that K-region dihydrodiols are by-products of the K-region arene oxide rearrangement. We know from other work that phenols are important metabolites of polycyclic arenes and that some phenols arise from the rearrangement of arene oxides.[3] Our studies have shown that carbocations are intermediates along the path from polycyclic arene oxides to phenols, and that these ions can react with the surrounding solvent. This means that in the cell intermediate carbocations from ring opening of metabolically produced arene oxides could react in a similar manner, but may also occasionally attack heteroatoms on nearby macromolecules. Thus, our results provide chemical evidence that arene oxides might bind to cell macromolecules by an SN1 process. Previous work on the reactions of benzene oxides has demonstrated facile SN2 attack of strong nucleophiles on the intact oxides, and SN1 reactions between sulfur nucleophiles and benzene oxide.[32]

Only the most nucleophilic atoms in cellular macromolecules would be expected to undergo SN2 reactions with arene oxides. However, since we have observed the reaction of carbocations with the less nucleophilic water molecule, it may be that arene oxides could also attach to less nucleophilic groups on cellular macromolecules. Such groups might include hydroxyl groups and phosphates in DNA[33] or RNA, and hydroxyl or amino groups in proteins. Future studies of the structures of arene oxide–macromolecule adducts should take this possibility into account.

Experimental Section

Materials. Reagent grade dioxane was purified by the method of Fieser.[34] It was mixed with an equal volume of double-distilled water 0.200 M in potassium chloride, sealed into 250-ml bottles under nitrogen, and stored at −20°. All other solvents were spectral quality or were distilled before use. Phenanthrene 9,10-oxide (**1**) and benz[*a*]anthracene 5,6-oxide (**2**) were prepared by the method of Newman and Blum;[35] 3-methylcholanthrene 11,12-oxide (**4**) and dibenz[*a,h*]anthracene 5,6-oxide (**3**) were prepared by the method of Sims.[15a,b]

Synthesis of *cis*-9,10-dihydro-9,10-dihydroxyphenanthrene (**5c**), *cis*-5,6-dihydro-5,6-dihydroxybenz[*a*]anthracene (**6c**), *cis*-5,6-dihydro-5,6-dihydroxydibenz[*a,h*]anthracene (**7c**), and *cis*-11,12-dihydro-11,12-dihydroxy-3-methylcholanthrene (**8c**) was by the method of Cook and Schoental.[16a] The trans dihydrodihydroxy compounds **5t**,[16b] **6t**,[15b] **7t**,[15b] and **8t**[15a] were prepared by lithium aluminum hydride–diethyl ether reduction of the respective *o*-quinones. The quinones were formed from the cis dihydrodiols by sodium dichromate oxidation in acetic acid.[15b] High-pressure liquid chromatography (see below) showed no contamination of trans dihydrodiols by the cis isomers.

The phenols **11** (**+10**), **14** (**+15**), and **19**, were synthesized by dehydration of the corresponding dihydrodiols (cis or trans) in acetic acid 1 M in hydrochloric acid at 100 °C.[15b] Cooling and addition of 1 vol of water, followed by centrifugation and several water washes, gave the slightly colored solids. These were air sensitive and had to be used immediately.

Spectra. Uv spectra were taken on a Cary 15 spectrophotometer with 1.00-cm microcells. Mass spectra were obtained with a Varian CH-7 spectrometer (Varian associates, Palo Alto, Calif.) equipped with a magnet-drive direct insertion probe (Variset Corp., Madison, Wis.). NMR spectra were recorded on a Bruker 90-MHz Fourier transform spectrometer locked on $CDCl_3$. Melting points were taken on a Thomas-Hoover apparatus and are corrected.

Thin Layer Chromatography (TLC). Eastman Kodak silica gel chromatogram sheets (No. 6060) were used.

3-Methylcholanthrene Oxide (4) in Dioxane–Water–H^+: Extraction of Phenols and Diols. To 1.0 ml of 1:1 dioxane–water, 0.10 M in KCl, at room temperature in a 15.0-ml centrifuge tube was added 14 μg (0.049 μmol) of **4** in 6.5 μl of tetrahydrofuran. A 0.25-ml aliquot was diluted to 1.00 ml and the uv spectrum was taken (A = 0.842 at 280 nm). The remaining solution was adjusted to pH 0 with concentrated hydrochloric acid, and another 0.25-ml aliquot was removed and diluted to 1.00 ml, and the uv spectrum was taken. The latter solution was combined with the original and mixed with 0.50 ml of hexane and 0.50 ml of 1 N aqueous potassium hydroxide. The mixture was vortexed, centrifuged at 1000 rpm, and separated. The hexane layer was extracted with 0.50 ml of 1 N potassium hydroxide and the combined aqueous layers were extracted with 0.50 ml of hexane. The uv spectrum of each layer was taken (A of hexane = 0.525 at 276 nm and A of aqueous = 0.461 at 301 nm); TLC of the hexane layer (9:1 cyclohexane–dioxane) showed one spot at R_f 0.045. The total amount of compound present in each solution was calculated either from the initial concentration or from the volume, dilution factor, and absorbance of the solution, and the known ϵ value at the specified wavelength.

There were only small differences in ϵ values between hexane and dioxane-water solutions. The percent recovery was calculated by the formula

$$\frac{(\mu\text{mol of dihydrodiol} + \mu\text{mol of phenol})100}{\mu\text{mol of epoxide}}$$

or

$$[(0.0106 + 0.0270)100]/[0.0495 - \tfrac{1}{4}(0.0495)] = 100\%;$$

The calculated percent yield of dihydrodiols was

$$\frac{(\mu\text{mol of dihydrodiol})100}{\mu\text{mol of epoxide}}$$

or

$$[(0.0160)100]/0.0376 = 28\%$$

Dibenz[*a,h*]anthracene Oxide (3) in Dioxane-Water-H^+: Extraction of Phenols and Diols. To 1.0 ml of 1:1 dioxane-water, 0.10 M in KCl, was added 14 μg of **3** (0.0477 μmol) in 6 μl of tetrahydrofuran. The solution was adjusted to pH 0 with concentrated hydrochloric acid, then mixed with 1.0 ml of hexane and 1.0 ml of 1 N potassium hydroxide. After separation, the organic layer was washed with 1.0 ml of 1 N potassium hydroxide, and the aqueous layer was washed with 1.0 ml of hexane. Absolute ethanol (1.0 ml) plus 0.1 ml of concentrated hydrochloric acid were added to the aqueous layer. The uv spectra were recorded (A of hexane = 0.808 at 281 nm; A of aqueous layer = 0.818 at 295 nm). The percent recovery was $[(0.010 + 0.045)100]/0.0477 = 115\%$. The calculated yield of dihydrodiols was $[(0.010)100]/[0.010 + 0.045] = 18\%$. TLC of the hexane layer (9:1 cyclohexane-dioxane) showed one spot at R_f 0.03.

Phenanthrene Oxide (1) in Dioxane-Water-H^+: Quantitative TLC. A sample of **1** (718 μg, 3.70 μmol) in 100 μl of tetrahydrofuran was added to 20 ml of 1:1 dioxane-water, 0.70 M in KCl, and the uv spectrum was taken; the solution was adjusted to pH 1 and the uv was taken again. Neutralization with sodium bicarbonate, solvent removal at 1 mm on a rotary evaporator, and chromatography on a silica gel TLC sheet (9:1 benzene-ethanol) gave two spots at R_f 0.3 and 0.5. These were extracted with 10.0 and 25.0 ml of 1:1 dioxane-water, respectively, and the uv's were taken. The R_f 0.3 solution had an absorbance of 0.723, at 269 nm with an ϵ of 1.58×10^4 M^{-1} cm^{-1}, equivalent to 0.46 μmol of 5,6-dihydrodiol. The R_f 0.5 solution had an absorbance of 2.50 at 248 nm with an ϵ of 3.24×10^4, equivalent to 1.93 μmol of 9-phenanthrol. The percent recovery was $[(0.46 + 1.93)100]/3.70 = 65\%$; the yield of dihydrodiols was $[(0.046)100]/(0.46 + 1.93) = 19\%$.

Computer Analysis of the Uv Spectra of Mixtures: ϵ Determinations. ϵ values were determined from uv spectra of **2, 3, 4, 6, 7, 8, 10 (+11), 14 (+15),** and **19** taken on a Cary 15 recording spectrophotometer (Figures 1-3) with 1:1 dioxane-water, 0.10 M in KCl as solvent. ϵ's were calculated at the maxima and minima of the phenols, dihydrodiols, and oxides. Several spectra were redetermined, and the deviations in ϵ were less than 1%.

Computer Analysis of Mixtures. Any mixture of phenols, dihydrodiols, and/or oxide could be analyzed by supplying the ϵ's of the individual compounds and the uv absorbances of the mixture at the same wavelengths (19 λ's for **2,** 26 λ's for **3,** 12 λ's for **4**). A multiple linear regression computer analysis[18] was then applied to obtain the molarity of each species in the solution. Table IV shows the results of computer analysis of two synthetic oxide-phenol-dihydrodiol mixtures.

Cis/Trans-Dihydrodiol Ratios by High-Pressure Liquid Chromatography (Table I). Samples of oxides **1, 2, 3,** and **4** (1 mg) were treated at room temperature with 25-ml portions of 1:1 dioxane-water, 0.10 M in KCl, at pH's 2.50, 2.67, 2.85, and 2.85, respectively. Also, 1-mg portions of **4** were allowed to react at pH's 3.0 and 9.0 at 37°. After about 15 min (6 h in the pH 9.0 reaction) each was neutralized with sodium bicarbonate, evaporated to dryness, and extracted with three 15-ml portions of methylene chloride. These solutions were concentrated at 10 mm on a rotary evaporator and several microliters (ca. 1 μg) was injected into a Du Pont high-pressure liquid chromatography apparatus, No. 830, equipped with a 250 × 0.65 cm Zorbax (silica gel) column at room temperature. The eluting solvent was 6% (v/v) (methylene chloride-2-propanol-acetic acid; 1000:20:0.1 (v/v)) in cyclohexane. Peaks were integrated by multiplying the peak height by peak width at half-height. Peak identity was established both by comparison of product retention times with those of synthetic dihydrodiols and by the uv spectra of eluted peaks.

Table IV. Computer Analysis of Synthetic Mixtures of K-Region Derivatives of Dibenz[*a,h*]anthracene and Benz[*a*]anthracene

Oxide	Soln	Mol % oxide[a]	Mol % phenols[a]	Mol % dihydrodiols[a]
3	1	75.5	18.8	5.82
		72.2	18.1	9.65
	2	60.4	29.8	9.74
		59.5	33.4	7.10
	3	45.5	40.9	13.6
		46.7	42.7	10.5
	4	14.7	64.3	20.9
		11.0	67.0	22.1
2	1	15.5	58.3	26.3
		16.6	57.3	26.1
	2	19.6	63.6	16.7
		21.4	61.3	17.2
	3	71.7	8.30	20.0
		74.9	8.64	16.5

[a] Top value for synthetic mixture; lower value calculated.

Boric Acid-Silica Gel Separation of 3-Methylcholanthrene Dihydrodiols (8c and 8t). TLC sheets were soaked for 5 min in 10% (w/v) boric acid-methanol, then air-dried for 1 h. Authentic samples of **8c** and **8t** were spotted and developed with 9:1 benzene-ethanol; R_f values were 0.365 and 0.268, respectively.

TLC Determination of 8c/8t Produced at Various pH's in Dioxane-Water. A sample (2 mg) of 3-methylcholanthrene oxide (**4**) was divided equally among three 25-ml portions of 1:1 dioxane-water, 0.10 M in KCl, at pH's 2.84, 3.96, and 4.90. After 20 min at room temperature, each was neutralized with sodium bicarbonate, the solvent was removed at 1 mm on a rotary evaporator, and the residue was extracted with two portions of diethyl ether. Concentrated ether solutions were applied to the origins of silica gel sheets and developed with 9:1 cyclohexane-dioxane. Dihydrodiol spots (R_f 0.05) were extracted, and the vacuum-concentrated extracts were chromatographed on boric acid-silica gel sheets (repeated developments with 9:1 benzene-ethanol); the spots at R_f 0.29 and 0.41 were extracted with diethyl ether, made up to 10.0 or 25.0 ml, respectively, and the absorptions at 276 nm were measured on the Cary 15.

TLC Determination of 8c/8t in Basic Dioxane-Water. A sample of **4** (1 mg) in 100 μl of tetrahydrofuran was added to 16 ml of 1:1 dioxane-water, 0.10 M in KCl, held at pH 11.0 and 47 °C under Cr^{2+}-scrubbed nitrogen[36] in a Radiometer pH stat. The uv spectrum showed very little oxide after 9 h. The solvent was removed on a rotary evaporator at 1 mm, the residue was extracted with three 25-ml portions of diethyl ether, and the diols were isolated by TLC as above to give two 5.00-ml dioxane-water solutions of **8c** and **8t,** respectively. Absorbances at 276 nm were 0.651 and 0.525, respectively; this corresponds to a 55:45 cis/trans ratio.

Control Experiment: 5c, 5t, 8c, and 8t at pH 2.40 in 1:1 Dioxane-Water-H^+. Samples (200 μg) of **5c, 5t, 8c,** and **8t** were each added separately to 25 ml of 1:1 dioxane-water, 0.10 M in KCl, at pH 2.40. After 20 min, each was neutralized with sodium bicarbonate, the solvent was removed by rotary evaporator, and the residue was chromatographed as usual on boric acid-silica gel sheets alongside authentic markers, to give very dark, well-separated diol spots with no stereoisomer mixtures visible.

Derivatization and Separation of Dibenz[*a,h*]anthracene Phenols (14 and 15). To 120 ml of 30% aqueous ethanol at pH 3.61 was added 600 μg (2.02 μmol) of **3** in 100 μl of tetrahydrofuran. After 10 min, the reaction was mixed with 150 ml of benzene and 200 ml of water and separated; the water extraction was repeated twice, and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporator, and 6 ml of ethereal diazomethane distilled from 2 g of Diazald[37] (Aldrich) was added. After 6 h, TLC (9:1 benzene-ethanol) showed no phenol (R_f 0.3) and a new spot at R_f 0.85. Elution with CH_2Cl_2 of the R_f 0.85 spot from a large-scale TLC, and injection of a concentrated

solution into a Packard U-column gas chromatograph (6 ft 10% OV-1 on Gas-Chrom-X, nitrogen carrier gas: inlet, 300°; column, 290°; detector, 340°) gave two peaks with the same retention times as authentic[19] ethers **16** and **17** (35 and 38 min, respectively). The **17:16** area ratio was 15 to 85.

Methoxy-3-methylcholanthrene after Methylation of Phenol from Acid-Catalyzed Rearrangement of Oxide 4. A 5.0-mg (0.018 mmol) sample of 3-methylcholanthrene 11,12-oxide dissolved in 50 μl of THF was added to 75 ml of 1:1 dioxane-water (v/v), 0.10 M in potassium chloride. The pH was adjusted to 3.0; after 10 min of stirring at room temperature the solution was neutralized with sodium bicarbonate and the solvent was removed under vacuum. The residue was treated with several 50-ml portions of ethereal diazomethane distilled from 2 g of Diazald.[37] After 24 h the solution was filtered and reduced under vacuum, and the residue was preparatively chromatographed on a 12 × 12 cm silica gel sheet (Kodak) eluted with 1:1 benzene-hexane. The R_f 0.70 strip was eluted and the solvent was removed under vacuum to yield 3 mg of yellowish solid. Uv λ_{max} (95% EtOH) (log ϵ) 225 (4.51), 262 (4.59), 284 (4.71), 294 (4.80), and 309 (4.50). NMR (90 MHz, $CDCl_3$) δ 2.43 (s, 3 H, 3-CH_3), 3.38 (m, 2 H, -CH_2-), 3.55 (m, 2 H, -CH_2-), 4.07 (s, 3 H, OCH_3), 6.84 (s, 1 H, H-12), 7.21-8.75 (m, 6 H, aryl), and 8.82 (s, 1 H, H-6). Ms *m/e* (relative abundance) 299 (24), 298 (100), 283 (13), 256 (11), 255 (49), 253 (10), 252 (13), 240 (19) and 239 (28).

Methanolysis of 3-Methylcholanthrene 11,12-Oxide (4). A 100-mg (0.35 mmol) sample of oxide **4** was dissolved in 200 ml of 35° methanol to which was added 10 ml of 2 N HCl. After 30 min at room temperature, the solvent was removed under vacuum and 250 ml of 1 N potassium hydroxide plus 200 ml of cyclohexane were added. The mixture was shaken and separated, and the organic layer was extracted with three 250-ml portions of 1 N potassium hydroxide to remove phenoxides. The organic layer was dried over anhydrous magnesium sulfate, the solvent removed under vacuum, and the 75-mg residue chromatographed on three 12 × 12 cm 250 μ silica gel plates (developed with 2% (v/v) methanol in benzene). The hydroxy ether strips were extracted giving 15 mg from the R_f 0.18 spot and 25 mg from R_f 0.35 spot. Ms (both spots same) *m/e* (rel abundance): 317 (26), 316 (100), 285 (39), 284 (59), 271 (26), 270 (17), 269 (27), 267 (20), 255 (37), 241 (20) and 240 (18). NMR (90 MHz, $CDCl_3$) R_f 0.18: δ 1.76 (d, 1 H, J = 6.5 Hz, OH), 2.41 (s, 3 H, 3-CH_3), 3.38 (s, 3 H, OCH_3), 3.45 (m, 4 H, -CH_2CH_2-), 4.70 (d, 1 H, J = 4 Hz, CHOMe), 4.85 (m, 1 H, CHOH), 7.2-7.9 (m, 6 H, aryl H), and 8.03 (s, 1 H, H-6). R_f 0.35: δ 2.41 (s, 3 H, 3-CH_3), 2.80 (d, 1 H, J = 12 Hz, OH), 3.29 (s, 3 H, OCH_3), 3.43 (m, 4 H, -CH_2CH_2-), 4.63 (d, 1 H, J = 4 Hz, CHOMe), 4.90 (q, 1 H, J = 3, 11 Hz, -CHOH), 7.25-7.95 (m, 6 H, aryl H), and 7.99 (s, 1 H, H-6). Uv λ_{max} (95% EtOH) (log ϵ) 254 (4.55), 266 (4.65), 276 (4.70), and 312 (4.00).

12-Methoxy-3-methylcholanthrene (21). To a solution of 9 mg (0.03 mmol) of *cis*-11,12-dihydro-11-hydroxy-12-methoxy-3-methylcholanthrene (**24**) in 2 ml of pyridine was added 4 mg of *p*-toluenesulfonyl chloride. After 4 h at room temperature, the reaction mixture was reduced under vacuum and separated by TLC on two 12 × 12 cm silica gel sheets eluted with benzene. The fluorescent R_f 0.73 bands were cut out and eluted with diethyl ether; solvent removal under vacuum gave 3.0 mg of solid (35%). Uv λ_{max} (95% EtOH) 226, 272, 289, and 302. Ms *m/e* (rel abundance) 299 (23), 298 (100), 283 (18), 265 (9), 255 (20), 253 (7), 252 (10), 241 (15), and 240 (21). NMR (90 MHz, $CDCl_3$) δ 2.44 (s, 3 H, 3-CH_3), 3.38 (m, 2 H, -CH_2), 3.98 (m, 2 H, -CH_2-), 4.04 (s, 3 H, OCH_3), 6.75 (s, 1 H, H-11), 7.25-7.83 (m, 5 H, aryl), 8.68 (m, 1 H, H-7), and 8.89 (s, 1 H, H-6).

5- and 6-Methoxybenz[*a*]anthracene (12 and 13). To a 100-ml flask equipped with reflux condenser, nitrogen inlet, heating mantle, and magnetic stirrer were added 200 mg (0.76 mmol) of *cis*-5,6-dihydro-5,6-dihydroxybenz[*a*]anthracene (**6c**), 50 ml of glacial acetic acid, and 2 ml of concentrated hydrochloric acid. The mixture was stirred at reflux for 30 min and cooled, and 50 ml of water was added. The precipitated phenols were filtered, dried under vacuum, and added to 50 ml of ethereal diazomethane distilled from 2 g of Diazald.[37] Several portions of diazomethane solution were added over 2 days at room temperature; then the solvent was removed under vacuum and the residue chromatographed on a 3 × 30 cm silica gel column packed and eluted with 1:1 (v/v) benzene-hexane. The single fluorescent band was collected and dried under vacuum; the yellowish solid weighed 125 mg (65% yield). λ_{max} (1:1 dioxane-water) 260 (s), 276 (s), 286, 296, 302 (s) and 325. NMR (90 MHz, $CDCl_3$) δ 4.09 (s, 3 H), 4.13 (s, 2 H), 6.85 (s, 0.66 H), 6.99 (s, 1 H), 7.27-8.43 (m, 14 H), 8.19 (s, 1 H), 8.84 (s, 0.66 H), 9.05 (s, 1 H), and 9.11 (s, 0.66 H). Ms *m/e* (rel abundance) 259 (18), 258 (79), 216 (19), 215 (100) and 213 (16).

5- and 6-Methoxybenz[*a*]anthracene-7,12-quinones (25 and 26). A sample of 5- and 6-methoxybenz[*a*]anthracene (**12** and **13**) (280 mg, 1.1 mmol) was dissolved in 280 ml of glacial acetic acid under N_2. A solution of 560 mg of sodium dichromate dihydrate (1.85 mmol) in 60 ml of glacial acetic acid was added with stirring over 15 min. The volume was reduced under vacuum to 100 ml, and 100 ml of water was added to give a yellow precipitate. The volume was further reduced to 150 ml and the solid was filtered and dried in air to give 280 mg of yellowish solid. TLC (benzene, alumina sheet) showed spots at R_f 0.82 (methoxybenz[*a*]anthracenes), 0.70 (methoxybenz[*a*]anthracenequinone), 0.42 (methoxybenz[*a*]anthracenequinone), and 0.24 (red impurity). Chromatography on a 2 × 30 cm alumina (CAMAG, neutral) column gave two yellow bands: the first was eluted between 300 and 800 ml of 1% ethyl acetate in benzene; the second (81 mg) was collected between 1000 and 1400 ml (43 mg). TLC showed some contamination of the second band by the first, so the second-band material was rechromatographed on a 2 × 9 cm silica gel-benzene column. Collection and solvent removal of the 20-60-ml fraction gave 6.5 mg of R_f 0.70 material; the 100-250-ml fraction gave 33 mg of R_f 0.42 material. The combined yield was 45%. Ms (R_f 0.42) *m/e* (rel abundance): 289 (22), 288 (100), 271 (16), 259 (44), 242 (16), 232 (21), 202 (26), and 189 (25). R_f 0.70: 289 (24), 288 (100), 273 (20), 259 (6), 245 (22), 231 (5), 217 (28), 202 (10), and 189 (32). NMR (90 MHz, $CDCl_3$; R_f 0.70: δ 4.16 (s, 3 H, OCH_3), 7.36-8.36 (m, 8 H, aryl), 9.70 (q, J = 2.2, 6.0 Hz, 1 H, H-1); R_f 0.42: δ 4.09 (s, 3 H, OCH_3), 7.35 (s, 1 H, H-5), 7.45-8.20 (m, 7 H, aryl H), and 9.36 (q, J = 3.5, 6 Hz, 1 H, H-1). Uv λ_{max} (95% EtOH) (log ϵ) R_f 0.70: 283 (4.72) and 297 (4.68); R_f 0.42: 255 (4.48) and 284 (4.36). Mp, R_f 0.70, 192-193°; R_f 0.42, 147-148°.

5-Methoxybenz[*a*]anthracene (12). A 38-mg (130 μmol) sample of methoxyquinone (R_f 0.70) was dissolved in 20 ml of distilled diglyme; 40 μl of distilled boron trifluoride etherate (320 μmol) and 16 mg of sodium borohydride (300 μmol) were added. After 2 h at room temperature, the diglyme was removed at 0.2 mm and the residue was chromatographed on a silica gel-benzene column (1 × 20 cm). Collection and removal of solvent from the fluorescent band gave 35 mg of whitish solid. Ms *m/e* (rel abundance) 259 (19), 258 (82), 216 (19), 215 (100), and 213 (17). NMR (90 MHz, $CDCl_3$) δ 4.11 (s, 3 H, OCH_3), 6.99 (s, 1 H, H-6), 7.25-8.85 (m, 8 H, aryl), 8.19 (s, 1 H, H-7), and 9.05 (s, 1 H, H-12). Uv λ_{max} (95% EtOH) (log ϵ) 223 (4.60), 258 (4.45), 278 (4.49), 285 (4.71), 301 (4.36), 319 (3.69), and 333 (3.74).

6-Methoxybenz[*a*]anthracene (13). To a solution of 3 mg (0.01 mmol) of R_f 0.42 methoxybenz[*a*]anthraquinone in 2 ml of dry tetrahydrofuran was added 10 mg of lithium aluminum hydride. The mixture was stirred at room temperature for 15 min, the solvent was removed under vacuum, and the residue was taken up in 10 ml each of benzene and 0.1 N HCl. The layers were separated and the organic layer was washed with two 10-ml portions of water. TLC of the residue (after solvent removal) on a 12 × 12 cm silica gel sheet developed with 1:1 benzene-hexane and extraction of the fluorescent band at R_f 0.5 gave 2.5 mg (80% yield) of **13.** Ms *m/e* (rel abundance) 259 (18), 258 (71), 216 (22), 215 (100), and 213 (18). Uv λ_{max} (95% EtOH) 225, 262, 275, 287, and 298. NMR (90 MHz, $CDCl_3$) δ 4.13 (s, 3 H, OCH_3), 6.88 (s, 1 H, H-5), 7.05-8.78 (m, 8 H, aryl), 8.85 (s, 1 H, H-7), and 9.11 (s, 1 H, H-12).

Rate Determinations. Oxide solvolysis reactions were run in a Radiometer pH stat which included a Radiometer TTTlc titrator equipped with a G2222C glass microelectrode, calomel reference electrode, and an SBR 2C Titrigraph, which drove a 0.50-ml syringe buret at the rate of 20 μl/min. The 17-ml reaction vessel was equipped with a magnetic stirring bar, thermostated jacket, and inlets for chromous-ion scrubbed nitrogen,[36] titrant, and electrodes. Water from a Haake Type FS3 circulating heater filled the vessel jacket, and the temperature was maintained at 36.8 ± 0.05 °C in the stirred solvent with a Bronwill Scientific mercury thermometer calibrated at 37.0° against a 29-41° thermometer (Brooklyn Thermometer Co., Farmingdale, N.Y.). Temperature variations

during a kinetic run were less than 0.05°. The electrodes were calibrated before a kinetic run at 36.8° with Matheson pH 4.03 ± 0.02 standard buffer in the acid range and Matheson pH 9.88 ± 0.02 standard buffer in the basic range. Then the vessel was rinsed with distilled water and the solvent, and was equilibrated at the same temperature. The pH was adjusted to the desired value with 1:1 dioxane–water containing potassium chloride (0.10 M) and hydrochloric acid from the buret. The solution in the buret varied in HCl concentration according to the pH of the reaction: 0.05 M at pH 3 to 0.002 M at pH 8. Potassium hydroxide in 1:1 dioxane–water (0.10 M in potassium chloride) was used to control the pH at pH's greater than 8; it varied from 0.001 M at pH 9 to 0.01 M at pH 12. At no time during any kinetic run did the pH vary more than 0.01 pH unit, nor was enough titrant added to increase the ionic strength more than 1%. If the pH was over 7, the solvent was purged with chromous ion scrubbed nitrogen[36] for 30 min prior to oxide addition, and a positive pressure of nitrogen was maintained during the reaction. The reaction commenced with addition of ca. 300 μg of arene oxide in 50 μl of tetrahydrofuran; this was enough material to give a uv absorbance of ca. 1 in a 0.50-ml aliquot diluted to 1.0 ml. Aliquots were removed at appropriate times (measured by stop.watch) with either a Pasteur pipet or an automatic pipet and were immediately quenched in 0.50 ml of 1:1 dioxane–water, 0.1 M potassium chloride, containing 10 mg of sodium bicarbonate at 0°. Time of quenching was less than 1 s.

Aliquots were analyzed by one of two methods: (a) for the reactions of dibenz[*a,h*]anthracene oxide (**3**) and 3-methylcholanthrene oxide (**4**), the uv spectrum (330–250 nm) of each quenched aliquot was taken on a Cary 15 spectrophotometer (it was not necessary to obtain accurate aliquot volumes because the computer program calculated the total concentration); (b) for the reaction of phenanthrene oxide (**1**) and benz[*a*]anthracene oxide (**2**), the absorption at one wavelength only (the λ_{max} of the product phenol, i.e., 248 nm for **9** and 290 nm for **10** (+**11**)) was measured on the Cary 15.

Analysis of the data from reactions of oxide **3** and **4** required first computing the mole percent of oxide, phenols, and dihydrodiols in each aliquot using the uv scan of each, the calculated ε's at several wavelengths, and a multiple linear regression computer program.[18] The mole percents and times of quenching were then submitted to a first-order rate constant program developed by J. W. Taylor.[38]

Analysis of the absorbance vs. time data of oxides **1** and **2** was also done with the Taylor computer program.[38] The points were weighted by the factor $(1/\text{absorbance})^2$ since the absorbance errors in the Cary 15 spectrophotometer are proportional to the absorbance.[39]

Calculation of Acid-Catalyzed Rate Coefficients, k_H. Each constant k_{obsd} (taken at pH less than 7) was divided by the $[H^+]$ (i.e., the negative antilog of the pH) in that reaction to give a k_H value, and the k_H values were averaged. The calculated relative standard errors of the rate constants for the reaction of oxides **1** and **2** were less than 1% of k_{obsd} and for those of oxides **3** and **4** were 1–4% of k_{obsd}; however, the estimated error in the pH determinations (±0.02 pH out of 4) was 4.5%. Thus, the relative error in k_H average was dominated by the pH determination error, and so the individual k_H's were weighted equally in the calculation of the average k_H.

Non-Acid-Catalyzed Rate Constant, k_0. This value was an average of the rate constants obtained at pH's greater than 8 weighted by the factor $(1/\text{relative standard error of rate constant})^2$.

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References and Notes

(1) J. A. Miller, *Cancer Res.*, **30**, 559 (1970).
(2) (a) T. Kuroki, E. Huberman, H. Marquardt, J. K. Selkirk, C. Heidelberger, P. L. Grover, and P. Sims, *Chem. Biol. Interact.*, **4**, 389 (1971–72); (b) P. Brookes, *Life Sci.*, **16**, 331 (1975).
(3) D. M. Jerina and J. W. Daly, *Science*, **185**, 573 (1974).
(4) J. Tomaszewski, D. M. Jerina, and J. W. Daly, in "Topics in Medicinal Chemistry", American Chemical Society, Washington, D.C., 1974, Chapter 30.
(5) (a) C. A. Coulson, *Adv. Cancer Res.*, **1**, 1 (1953); (b) A. Pullman and B. Pullman, *ibid.*, **3**, 117 (1955).
(6) P. L. Grover, A. Hewer, and P. Sims, *FEBS Lett.*, **18**, 76 (1971).
(7) J. K. Selkirk, E. Huberman, and C. Heidelberger, *Biochem. Biophys. Res. Commun.*, **43**, 1010 (1971).
(8) (a) P. L. Grover, P. Sims, E. Huberman, H. Marquardt, T. Kuroki, and C. Heidelberger, *Proc. Nat. Acad. Sci. U.S.A.*, **68**, 1098 (1971); (b) H. Marquardt, T. Kuroki, E. Huberman, J. K. Selkirk, C. Heidelberger, P. L. Grover, and P. Sims, *Cancer Res.*, **32**, 716 (1972); (c) E. Huberman, T. Kuroki, H. Marquardt, J. K. Selkirk, C. Heidelberger, P. L. Grover, and P. Sims, *ibid.*, **32**, 1391 (1972); (d) H. Marquardt, *ibid.*, **34**, 1612 (1974).
(9) E. Huberman, L. Aspiras, C. Heidelberger, P. L. Grover, and P. Sims, *Proc. Nat. Acad. Sci., U.S.A.*, **68**, 3195 (1971).
(10) W. M. Baird and P. Brookes, *Cancer Res.*, **33**, 2378 (1973).
(11) G. J. Kasperek and T. C. Bruice, *J. Am. Chem. Soc.*, **94**, 198 (1972).
(12) G. J. Kasperek, T. C. Bruice, H. Yagi, and D. M. Jerina, *J. Chem. Soc., Chem. Commun.*, 784 (1972).
(13) G. J. Kasperek, T. C. Bruice, H. Yagi, N. Kaubisch, and D. M. Jerina, *J. Am. Chem. Soc.*, **94**, 7876 (1972).
(14) P. Y. Bruice, T. C. Bruice, H. Selander, H. Yagi, and D. M. Jerina, *J. Am. Chem. Soc.*, **96**, 6814 (1974).
(15) (a) P. Sims, *Biochem. J.*, **98**, 215 (1966); (b) E. Boyland and P. Sims, *ibid.*, **97**, 7 (1965).
(16) (a) J. W. Cook and R. Schoental, *J. Chem. Soc.*, 170 (1948); (b) E. Booth, E. Boyland, and R. Turner, *ibid.*, 1188 (1950).
(17) A mixture of 5- and 6-methoxybenz[*a*]anthracenes has been isolated from the methylation of phenols produced from acid-catalyzed rearrangement of benz[*a*]anthracene oxide (**2**). Also the dehydration of the benz[*a*]anthracene K-region dihydrodiol produced after methylation a 3:1 mixture of 5- and 6-methoxybenz[*a*]anthracenes. See R. G. Harvey, S. Goh, and C. Cortez, *J. Am. Chem. Soc.*, **97**, 3468 (1975).
(18) REGAN2 is a statistics package program written by J. R. Allen for the University of Wisconsin Academic Computing Center, Madison, Wis.
(19) J. A. LaBudde and C. Heidelberger, *J. Am. Chem. Soc.*, **80**, 1225 (1958).
(20) K. D. Bartle, D. W. Jones, and R. S. Matthews, *Spectrochim. Acta, Part A*, **25**, 1603 (1969).
(21) A. J. Swaisland, P. L. Grover, and P. Sims, *Biochem. Pharmacol.*, **22**, 1547 (1973).
(22) L. Hammett, "Physical Organic Chemistry", McGraw-Hill, New York, N.Y., 1970, p 317.
(23) All monohydro or dihydrobenzo rings illustrated as planar are probably equilibrating pairs of nonplanar conformers. See P. W. Rabideau and R. G. Harvey, *J. Chem. Soc., Chem. Commun.*, 1005 (1969).
(24) (a) Reference 22, p 323; (b) D. L. Whalen and A. M. Ross, *J. Am. Chem. Soc.*, **96**, 3678 (1974).
(25) This argument assumes that the isomeric benzylic cations give similar dihydrodiol/phenol ratios. This is probably a safe assumption since the dihydrodiol/phenol ratios for oxides **1–4** remain essentially constant in spite of nearly 100-fold differences in rates of formation of the intermediate ions.
(26) H. C. Longuet-Higgins, *J. Chem. Phys.*, **18**, 265, 275, 283 (1950).
(27) (a) F. Dewhurst and J. Orozco, *Br. J. Cancer*, **29**, 92 (1974); (b) J. W. Daly, D. M. Jerina, and B. Witkop, *Experientia*, **28**, 1129 (1972).
(28) C. Battistini, A. Balsamo, G. Berti, P. Crotti, B. Macchia, and F. Macchia, *J. Chem. Soc., Chem. Commun.*, 712 (1974).
(29) There does appear to be a temperature effect on the cis/trans dihydrodiol product ratio (see C. Battistini, P. Crotti, and F. Macchia, *Tetrahedron Lett.*, 2091 (1975)). However, the small effect seen in the solvolysis of oxide **4** at pH 3.0 at 25 and 37° (Table I) would predict a 69:31 cis/trans dihydrodiol ratio at 47 °C (by plotting $1/T$ vs. log (cis/trans) as in Battistini et al.). Thus, the 55:45 cis/trans dihydrodiol ratio is significantly lower.
(30) R. B. Woodward and R. Hoffmann, "Conservation of Orbital Symmetry", Springer-Verlag, New York, N.Y., 1970, Chapter 7.
(31) E. Heilbronner, "Hückel Molecular Orbitals", Springer-Verlag, West Berlin, 1966.
(32) (a) A. M. Jeffrey, H. Yeh, D. M. Jerina, R. M. DeMarinis, C. H. Foster, D. E. Piccolo, and G. A. Berchtold, *J. Am. Chem. Soc.*, **96**, 6929 (1974); (b) D. M. E. Reuben and T. C. Bruice, *J. Chem. Soc., Chem. Commun.*, 113 (1974).
(33) (a) P. D. Lawley has found that compounds which react by an SN1 mechanism, e.g., *N*-methyl-*N*-nitrosourea, methylate DNA partially on phosphate: P. D. Lawley in "Topics in Chemical Carcinogenesis", W. Nakahara, S. Takayama, T. Sugimura, and S. Odashima, Ed., University of Tokyo Press, Tokyo, 1972, p 237. (b) P. B. Hulbert, *Nature (London)*, **256**, 146 (1975).
(34) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. I, Wiley, New York, N.Y., 1967, p 333.
(35) M. S. Newman and S. Blum, *J. Am. Chem. Soc.*, **86**, 5598 (1964).
(36) D. F. Shriver, "Manipulation of Air-Sensitive Compounds", McGraw-Hill, New York, N.Y., 1969, p 139.
(37) Reference 34, p 191.
(38) R. C. Williams and J. W. Taylor, *J. Chem. Educ.*, **47**, 129 (1970).
(39) "Model 15 Recording Spectrophotometer Instruction Manual", Cary Instruments, Monrovia, Calif., p 14.