irradiated for 10 days. Examination by ^{1}H NMR spectroscopy of the residue after workup showed 30% products. The residue was separated by silica gel TLC, giving two bands. The less polar band (392 mg) was starting material **6** and [3.2.1] dichlorides in a ratio of 3.9:1. The more polar band (188 mg) was [3.2.1] acetates.

The [3.2.1] dichloride band was heated at reflux with silver perchlorate in 50:50 acetone:water mixture for 2 min. The [3.2.1] alcohols were separated from the [2.2.2] dichlorides by silica gel TLC. After oxidation of the [3.2.1] alcohols (82 mg), the ketone products were **25** and **27** in a ratio of 2.6:1.

The [3.2.1] acetates were converted to a ketone mixture of **25** and **27** in a ratio of 4.7:1.0.

The irradiation of **6** at 300 nm was repeated in a heavy-walled Pyrex tube. The irradiated sample required 45 days to go to 40% conversion, and no significant difference in products was found.

In all the irradiations of **6** a small amount of **23-X** was observed, but the relative amount of **23** was time-dependent. Short irradiation samples showed 2-3%, whereas long irradiation samples showed 10-15%. This is indicative of **23** being a secondary product.

Irradiation of 6 in Glacial Acetic Acid at 254 nm. Compound **6** (439 mg, 1.44 mmol) was dissolved in 110 mL of HOAc and irradiated for 48 h in the 254-nm Rayonet. After workup, the residue showed no starting material, and only [3.2.1] acetate products were visible. The products were **17**-OAc, **19**-OAc, and **23**-OAc in a ratio of 5:1:1, as measured by ^{1}H NMR spectroscopy of the acetate mixture.

Acknowledgment. We are indebted to the National Science Foundation (Grants CHE-80-11933 and CHE-85-03422) and to the Office of Basic Energy Sciences, U.S. Department of Energy (Contract AC02-79ER10366) for support of this research.

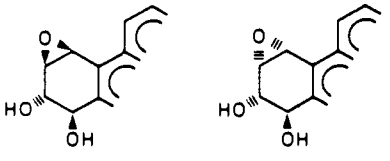
Effects of a 6-Fluoro Substituent on the Solvolytic Properties of the Diastereomeric 7,8-Diol 9,10-Epoxides of the Carcinogen Benzo[*a*]pyrene

H. Yagi,[†] J. M. Sayer,[†] D. R. Thakker,[†,‡] W. Levin,[§] and D. M. Jerina*[†]

Contribution from the Section on Oxidation Mechanisms, Laboratory of Bioorganic Chemistry, NIDDK, The National Institutes of Health, Bethesda, Maryland 20892, and the Laboratory of Experimental Carcinogenesis and Metabolism, Roche Institute of Molecular Biology, Nutley, New Jersey 07110. *Received July 21, 1986*

Abstract: 6-Fluorinated analogues of the mutagenic and carcinogenic benzo[*a*]pyrene 7,8-diol 9,10-epoxides have been synthesized by epoxidation of metabolically formed (−)-*trans*-(7*R*,8*R*)-7,8-dihydroxy-7,8-dihydro-6-fluorobenzo[*a*]pyrene to produce (7*R*,8*S*,9*R*,10*S*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (**1a**) and (7*R*,8*S*,9*S*,10*R*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (**2a**). NMR spectra indicate that the 7,8-diol group of **1a** is almost exclusively pseudoaxial whereas the diol group in **2a** prefers the pseudoaxial orientation to a lesser extent. In both cases the preference for the pseudoaxial conformation of the diol group is much stronger in the fluorinated diol epoxides than in the corresponding benzo[*a*]pyrene derivatives. Like the benzo[*a*]pyrene diol epoxides, **1a** and **2a** undergo hydrolysis and rearrangement in aqueous solutions to give tetraols and a 9-keto 7,8-diol, according to the rate law $k_{obsd} = k_H a_{H^+} + k_0$. Studies with 9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (**3a**) and its corresponding benzo[*a*]pyrene derivative **3b** indicated that the electronic effect of the 6-fluoro group decreases k_H by ~7-fold and k_0 by ~11-fold. Relative magnitudes of k_H for the fluorinated and unfluorinated diol epoxides can be accounted for solely by this electronic effect. On the other hand, k_0 for **1a** is much smaller and k_0 for **2a** is much larger than predicted when only the electronic substituent effect of fluorine is considered. The pH-independent rates for solvolysis of the fluorinated diol epoxides are thus markedly affected by their altered conformational equilibria due to the presence of fluorine. The observed differences in conformation of the fluorinated diol epoxides may account for the reduced mutagenicity of **1a** and **2a** as well as the lack of high tumorigenicity for **2a** relative to their benzo[*a*]pyrene counterparts, since bay-region diol epoxides in which the hydroxyl groups prefer the pseudoaxial conformation are known not to be highly carcinogenic.

Diol epoxides whose epoxide group forms part of a bay region are the only known metabolically formed ultimate carcinogens from the polycyclic aromatic hydrocarbons.[1] Since such diol epoxides are formed from *trans*-dihydrodiols, two diastereomers are possible, in which the benzylic hydroxyl group and the epoxide



diastereomer-1 diastereomer-2

oxygen are cis (diastereomer 1) or trans (diastereomer 2). In the absence of specific structural features, the benzo-ring *trans*-dihydrodiol precursors markedly prefer the conformation in which the hydroxyl groups are pseudoequatorial.[2] Although this conformational preference is maintained for the derived diol epoxide 2 diastereomers, the diol epoxide 1 diastereomers have a small preference for the conformation in which the hydroxyl groups are

[†] National Institutes of Health.
[§] Roche Institute of Molecular Biology.
[‡] Present address: Center for Drugs and Biologies, FDA.

(1) (a) Levin, W.; Wood, A.; Chang, R.; Ryan, D.; Thomas, P.; Yagi, H.; Thakker, D.; Vyas, K.; Boyd, C.; Chu, S.-Y.; Conney, A.; Jerina, D. *Drug Metab. Rev.* **1982**, *13*, 555-580. (b) Nordqvist, M.; Thakker, D. R.; Yagi, H.; Lehr, R. E.; Wood, A. W.; Levin, W.; Conney, A. H.; Jerina, D. M. In *Molecular Basis of Environmental Toxicity*; Bhatnagar, R. S., Ed.; Ann Arbor Science Publishers: Ann Arbor, MI, 1980; pp 329-357. (c) Thakker, D. R.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H.; Jerina, D. M. In *Bioactivation of Foreign Compounds*; Anders, M. W., Ed.; Academic: Orlando, FL, 1985; pp 177-242. (d) Dipple, A.; Moschel, R. C.; Bigger, C. A. H. In *Chemical Carcinogens*; Searle, C. E., Ed.; American Chemical Society: Washington, D.C., 1984; ACS Monograph 182, pp 41-163.

(2) Jerina, D. M.; Selander, H.; Yagi, H.; Wells, M. C.; Davey, J. F.; Mahadevan, V.; Gibson, D. T. *J. Am. Chem. Soc.* **1976**, *98*, 5988-5996. Zacharias, D. E.; Glusker, J. P.; Fu, P. P.; Harvey, R. G. *J. Am. Chem. Soc.* **1979**, *101*, 4043-4051.

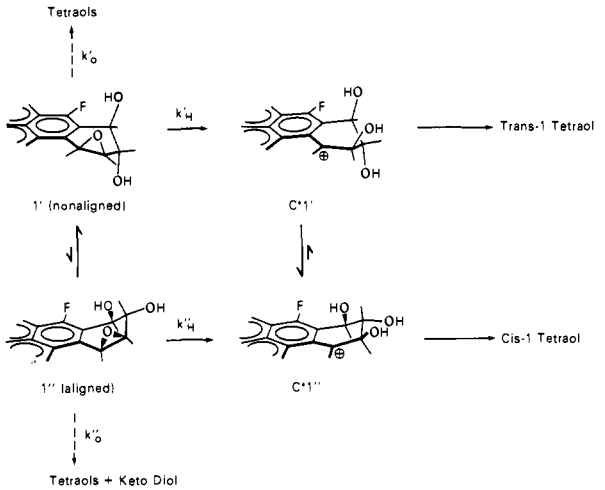


Figure 1. Conformational equilibria of diol epoxide 1 diastereomers and their effect on the hydrolysis products observed under acidic (k_H) and pH-independent (k_0) conditions. The major pathways for trapping by water of hypothetical cationic intermediates in the k_H reaction are assumed to involve pseudoaxial approach of water. For the k_0 reaction, mechanistic pathways are not shown; the keto diol rearrangement product is presumed to arise only from the aligned conformer or the zwitterion derived from it (cf. ref 5 and 14). In the case of **1a** the equilibrium between the two conformations of the epoxide strongly favors 1′.

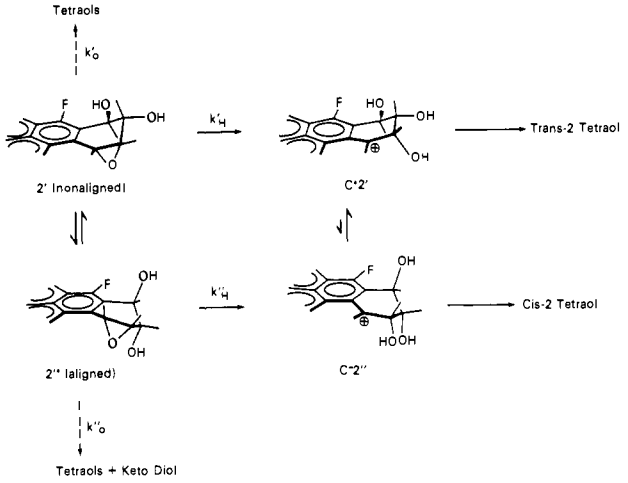


Figure 2. Conformational equilibria of diol epoxide 2 diastereomers and their effect on the hydrolysis products observed under acidic (k_H) and pH-independent (k_0) conditions. The major pathways for trapping by water of hypothetical cationic intermediates in the k_H reaction are assumed to involve pseudoaxial approach of water. For the k_0 reaction, mechanistic pathways are not shown; the keto diol rearrangement product is presumed to arise only from the aligned conformer or the zwitterion derived from it (cf. references 5 and 14). In the case of **2a** the equilibrium between the two conformations of the epoxide slightly favors 2″.

pseudoaxial.[3] Tumor studies of such bay-region diol epoxides derived from carcinogenic hydrocarbons have shown that diastereomer 2 diol epoxides have relatively high tumorigenic activity whereas diastereomer 1 diol epoxides are inactive.[4] Conformational factors play an important role in the reactivity of diol epoxides in pH-independent solvolysis reactions, such that the conformation in which the C–O bond that is cleaved is aligned with the π orbitals of the aromatic rings reacts more rapidly than does the conformation in which this C–O bond is not well aligned with the π system.[3c,5] In the isomer 1 series, this reactive aligned conformation (cf. Figure 1, 1″) has pseudoequatorial hydroxyl groups, whereas in the isomer 2 series, which has the opposite relative stereochemistry of the epoxide and hydroxyl groups, the aligned conformation of the epoxide (cf. Figure 2, 2″) has pseudoaxial hydroxyl groups. For "normal" diol epoxides, the more reactive conformers are not the preferred conformers for either diastereomer.

Several structural features that are capable of altering the normal, pseudoequatorial conformation of benzo-ring dihydrodiols have been identified. If the dihydrodiol forms part of a bay region, as in the case of the 1,2-dihydrodiol derived from benz[*a*]anthracene, steric hindrance between the benzylic hydroxyl group and the aromatic ring hydrogen across the bay region causes the hydroxyl groups to prefer the pseudoaxial conformation.[2] The importance of this bay-region hydrogen is illustrated by the fact that when it is absent, as in the 1,2-dihydrodiols of benz[*c*]acridine[6] and dibenz[*c*,*h*]acridine,[7] the pseudoequatorial conformation is preferred. The presence of a bulky substituent such as a bromine or a methyl peri to the benzylic hydroxyl group can cause the dihydrodiol to be pseudoaxial.[8] Finally, electrostatic effects from a fluorine[9,10] or a carbonyl group[11] can shift the equilibrium toward pseudoaxial hydroxyl groups. In the present study we have used the metabolically formed pseudoaxial (7*R*,8*R*)-dihydrodiol of 6-fluorobenzo[*a*]pyrene[9] to synthesize diol epoxide 1 and 2 diastereomers so that their chemical and biochemical properties could be compared to those of diol epoxides derived from benzo[*a*]pyrene. Our intention was to prepare a benzo[*a*]pyrene diol epoxide 2 diastereomer that had pseudoaxial hydroxyl groups, yet whose parent hydrocarbon had essentially identical steric requirements when compared with the carcinogenic benzo[*a*]pyrene. Since the van der Waals radius of fluorine (1.35 Å) is very close to that for hydrogen (1.2 Å), the use of a 6-fluoro substituent provided a means for achieving this end.

Results and Discussion

Synthesis and Conformational Analysis of 6-Fluorobenzo[*a*]pyrene 7,8-Diol 9,10-Epoxides 1a and 2a. We have previously noted that benzo-ring dihydrodiols with pseudoequatorial hydroxyl groups undergo highly stereoselective peroxyacid epoxidation to give only diastereomer 2 diol epoxides.[3] However, when the hydroxyl groups of the dihydrodiol prefer the pseudoaxial conformation, as is the case for the bay-region 1,2-dihydrodiol of triphenylene, the 9,10-dihydrodiol of benzo[*a*]pyrene, and the 9,10-dihydrodiol of benzo[*e*]pyrene, this high stereoselectivity[3] is lost and peroxyacid oxidation gives a mixture of diastereomer 1 and 2 diol epoxides.[12] Epoxidation (*m*-chloroperoxybenzoic acid in tetrahydrofuran) of (−)-**4**, the metabolically formed[13] (−)-(7*R*,8*R*)-dihydrodiol of 6-fluorobenzo[*a*]pyrene (6-FBP) whose hydroxyl groups are also predominantly in the pseudoaxial con-

(3) (a) Yagi, H.; Thakker, D. R.; Hernandez, O.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 1604–1611. (b) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *Tetrahedron Lett.* **1977**, 539–542. (c) Whalen, D. L.; Ross, A. M.; Yagi, H.; Karle, J. M.; Jerina, D. M. *J. Am. Chem. Soc.* **1978**, *100*, 5218–5221. Epoxidation of allylic cyclohexenols is known to occur with high cis stereoselectivity when the hydroxyl group is predominantly pseudoequatorial: Henbest, H. B. *Proc. Chem. Soc., London* **1963**, 159–165.

(4) Reviewed in the following: Jerina, D. M.; Sayer, J. M.; Agarwal, S. K.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H.; Pruess-Schwartz, D.; Baird, W.; Pigott, M. A.; Dipple, A. In *Biological Reactive Intermediates-III*; Kocsis, J. J., Jollow, D. J., Witmer, C. M., Nelson, J. O., Snyder, R., Eds.; Plenum: New York, 1986; pp 11–30.

(5) Sayer, J. M.; Yagi, H.; Silverton, J. V.; Friedman, S. L.; Whalen, D. L.; Jerina, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 1972–1978.

(6) Lehr, R. E.; Kumar, S. *J. Org. Chem.* **1981**, *46*, 3675–3681.

(7) Lehr, R. E.; Kumar, S.; Shirai, N.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 98–107.

(8) Yang, S. K.; Fu, P. P. *Chem.-Biol. Interact.* **1984**, *49*, 71–88. Fu, P. P.; Yang, S. K. *Carcinogenesis* **1983**, *4*, 979–984.

(9) Buhler, D. R.; Unlu, F.; Thakker, D. R.; Slaga, T. J.; Conney, A. H.; Wood, A. W.; Chang, R. L.; Levin, W.; Jerina, D. M. *Cancer Res.* **1983**, *43*, 1541–1549.

(10) Chiu, P.-L.; Fu, P. P.; Yang, S. K. *Cancer Res.* **1984**, *44*, 562–570.

(11) Vyas, K. P.; Shibata, T.; Highet, R. J.; Yeh, H. J.; Thomas, P. E.; Ryan, D. E.; Levin, W.; Jerina, D. M. *J. Biol. Chem.* **1983**, *258*, 5649–5659.

(12) (a) Yagi, H.; Thakker, D. R.; Lehr, R. E.; Jerina, D. M *J. Org. Chem.* **1979**, *44*, 3439–3442. (b) Harvey, R. G.; Lee, H. M.; Shyamasundar, N. *J. Org. Chem.* **1979**, *44*, 78–83. (c) Thakker, D. R.; Yagi, H.; Lehr, R. E.; Levin, W.; Buening, M.; Lu, A. Y. H.; Chang, R. L.; Wood, A. W.; Conney, A. H.; Jerina, D. M. *Mol. Pharmacol.* **1978**, *14*, 502–513.

(13) Thakker, D. R.; Yagi, H.; Sayer, J. M.; Kapur, U.; Levin, W.; Chang, R. L.; Wood, A. W.; Conney, A. H.; Jerina, D. M. *J. Biol. Chem.* **1984**, *259*, 11249–11256.

Table I. ^{1}H NMR Spectra (100 MHz, Me_2SO-d_6 for **1a** and **2a** and Acetone-d_6 for **1c** and **2c**) of the Diastereomeric Benzo-Ring 7,8-Diol 9,10-Epoxides (**1a**, **2a**) and 9,10-Diol 7,8-Epoxides (**1c**, **2c**) from 6-FBP after Exchange with CD_3OD

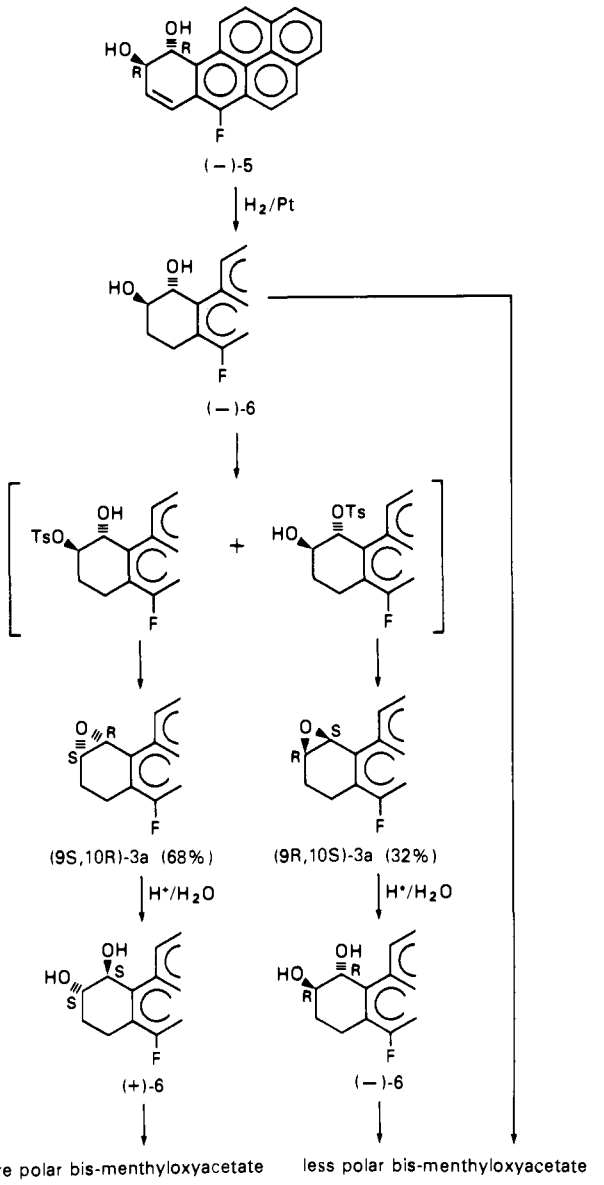
	oxirane protons		carbinol protons		
diol epoxides	benzylic	nonbenzylic	benzylic	nonbenzylic	aromatic protons
	H_{10}	H_9	H_7	H_8	
1a	5.24	4.02	5.16	4.50	8.0–8.5 (6 H), H_{11} 8.78
		($J_{7,8} = J_{8,9} = J_{7,9} = 2.5$, $J_{9,10} = 4.0$, $J_{F_6,10} = 1.7$)[a]			($J_{11,12} = 10$)
2a	5.08	3.94	4.92	4.20	8.0–8.5 (6 H), H_{11} 8.64
		($J_{7,8} = 5.5$, $J_{8,9} = 3.5$, $J_{9,10} = 4.0$, $J_{F_6,10} = 1.8$)[b]			($J_{11,12} = 10$)
	H_7	H_8	H_{10}	H_9	
1c	4.82	4.20	5.58	4.80	7.9–8.4 (6 H), H_{11} 8.44
		($J_{7,8} = 4.0$, $J_{8,9} = J_{8,10} = J_{9,10} = 2.5$)			($J_{11,12} = 10$)
2c	4.70	4.00	5.38	4.36	7.9–8.4 (6 H), H_{11} 9.04
		($J_{7,8} = 4.0$, $J_{8,9} = 2.5$, $J_{9,10} = 6.5$)			($J_{11,12} = 10$)

[a] Before exchange, OH_7 4.54 and OH_8 5.50 δ with $J_{7,OH_7} = 8.5$ and $J_{8,OH_8} = 5.0$ Hz. [b] Before exchange, OH_7 5.26 and OH_8 5.84 δ with $J_{7,OH_7} = 5.3$ and $J_{8,OH_8} = 6.0$ Hz.

formation,[9] resulted in the formation of a mixture containing nearly equal amounts of the two diastereomeric diol epoxides, **1a** and **2a**. ^{1}H NMR spectra are given in Table I. Electrostatic repulsion between the 6-fluoro and the 7-hydroxyl group, in addition to the steric constraints imposed by unfavorable interaction between the 8-hydroxyl group and hydrogen at C_9 when the hydroxyl groups are pseudoequatorial, forces the fluorinated diol epoxide **1a** (less polar isomer on HPLC) into a conformation in which the hydroxyl groups take almost exclusively the pseudoaxial conformation with $J_{7,8} = 2.5$ Hz (Figure 1, 1′). For the fluorinated compound, this conformational preference is much stronger than for the unfluorinated diol epoxide 1 of benzo[*a*]pyrene (**1b**), for which $J_{7,8} = 6.0$ Hz. Similar small values of J_{diol} for diastereomer 1 isomers (1.7–2.5 Hz) have been observed previously for diol epoxides in which the diol groups are in a bay region.[12,14] The observation of a long-range ^4J "W" coupling between the benzylic proton, H_7, and the coplanar non-benzylic oxirane proton, H_9 ($J_{7,9} = 2.5$ Hz), which is only possible for a diol epoxide 1 diastereomer in the conformation with pseudoaxial hydroxyl groups, also requires that this conformation (Figure 1, 1′) be predominant for **1a**. Electronic repulsion between fluorine at C_6 and the hydroxyl group at C_7 affects the fluorinated diol epoxide **2a** (more polar isomer) in a similar manner. The observed value of $J_{7,8} = 5.5$ Hz for **2a** indicates that its hydroxyl groups have a greater preference for the pseudoaxial conformation (Figure 2, 2″) than do the hydroxyl groups in its benzo[*a*]pyrene counterpart, **2b** ($J_{7,8} = 9$ Hz). This preference for axial hydroxyl groups is not as strong as that observed in the case of diol epoxides in the diastereomer 2 series with both the benzylic hydroxyl group and the epoxide group in bay regions ($J_{diol} \sim 3.5$ Hz).[12a,b] Another interesting feature in the ^{1}H NMR spectra of the fluorinated diol epoxides is a long-range coupling between fluorine and H_{10} ($J = 1.7$–1.8 Hz) observed for both **1a** and **2a**.

Synthesis of 9,10-Epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (3a). The fluorine substituent at C_6 could affect the chemical reactivity of the diol epoxides by an inductive effect on the stability of a carbocation at C_{10} as well as by alteration of their preferred conformations. In order to separate these two effects, the fluorinated tetrahydroepoxide, **3a**, was synthesized (Scheme I). The metabolically obtained[9,13] (−)-(9*R*,10*R*)-dihydrodiol of 6-FBP, (−)-**5**, was hydrogenated over platinum to give quantitatively the (−)-6-fluorotetrahydro 9,10-diol, (−)-**6**. A convenient one-pot procedure for the formation of the 6-fluorotetrahydro 9,10-epoxide, **3a**, from (−)-**6** involved cyclization of its mixed monotosylates with sodium hydride. The extent of tosylation at either hydroxyl group would be reflected by the enantiomeric composition of the resultant tetrahydroepoxide **3a** (Scheme I). Acid-catalyzed hydrolysis of **3a** gave a mixture of *trans*- and *cis*-tetrahydrodiols by attack of water at the more reactive benzylic C_{10} position. The *trans*-tetrahydrodiol was converted to its diastereomeric diesters with the acid chloride of

Scheme I



(−)-menthyloxyacetic acid; less polar ($k' = 1.9$) and more polar ($k' = 2.6$) diastereomers were formed in a ratio of 32 to 68. The configurations shown in Scheme I were deduced as follows. The metabolically formed[9,13] *trans*-9,10-dihydrodiol of 6-FBP, (−)-**5**, gives a single diastereomer (less polar isomer) upon reduction and formation of the bis(menthyloxyacetyl) ester. Thus, metabolically formed **5** consists *entirely* of a single enantiomer, (−)-**5**, whose absolute configuration had previously been assigned on the basis

(14) Sayer, J. M.; Whalen, D. L.; Friedman, S. L.; Paik, A.; Yagi, H.; Vyas, K. P.; Jerina, D. M. *J. Am. Chem. Soc.* **1984**, *106*, 226–233.

Table II. ^{1}H NMR Spectra ($CDCl_3$) of Tetraol Tetraacetates Derived from 6-FBP 7,8-Diol 9,10-Epoxides

compound	methine protons H_7	H_8	H_9	H_{10}	aromatic protons	acetyl methyl
cis-1 tetraol tetraacetate[a]	6.88	6.00 ($J_{7,8}$ = 6.0, $J_{8,9}$ = 11.5, $J_{9,10}$ = 3.5)	5.42	7.34	7.9–8.4 (7 H)	2.12, 2.15 2.17 (×2)
trans-2 tetraol tetraacetate[b]	6.94	5.79 ($J_{7,8}$ = 7.27, $J_{8,9}$ = 2.5, $J_{9,10}$ = 3.6)	5.79	6.97	8.0–8.3 (7 H)	2.00, 2.10 2.17, 2.20
cis-2 tetraol tetraacetate[a]	6.66	5.58 ($J_{7,8}$ = 3.0, $J_{8,9}$ = 2.3, $J_{9,10}$ = 4.5)	5.75	7.19	8.0–8.5 (7 H)	1.99 (×2) 2.01 (×2)

[a] Spectrum at 100 MHz. [b] Spectrum at 500 MHz.

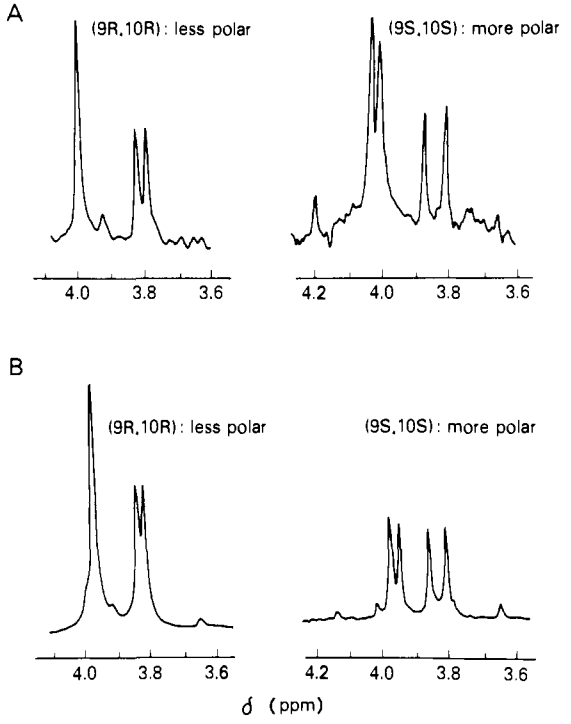


Figure 3. Partial ^{1}H NMR spectra (100 MHz, benzene-d_6) of the bis-((−)-menthyloxyacetic) acid esters of (A) the (+)- and (−)-*trans*-9,10-diols of 7,8,9,10-tetrahydro-6-FBP and (B) the (+)- and (−)-*trans*-9,10-diols of 7,8,9,10-tetrahydro-BP. When the trans *O*-menthyloxycarbonyl groups are attached to carbon atoms with (*S*,*S*) absolute configuration (more polar diastereomers), the diastereotopic exocyclic methylene hydrogens (H_A and H_B in $-OCH_AH_BCO_2-$) are nonequivalent and appear as a pair of doublets.

of CD spectra[13] as (9*R*,10*R*), and the less polar bis(menthyloxy)acetate, by correlation with this known compound, must also have (9*R*,10*R*) absolute configuration. The absolute configurations of these diastereomeric esters were confirmed by examination of the ^{1}H NMR spectra of their diastereotopic exocyclic methylene protons H_A and H_B in the $-OCH_AH_BCO_2-$ portion (Figure 3). Previous studies of the bis(menthyloxyacetyl) esters of *trans*-diol derivatives on saturated benzo rings of polycyclic aromatic hydrocarbons have shown that the diastereotopic $-CH_2-$ protons in the pair of $-OCH_2CO_2-$ groups of the less polar (*R*,*R*) bis-esters appear as two singlets or a singlet and a weakly split AB quartet, whereas the more polar (*S*,*S*)-diastereosomers show these protons as a pair of AB quartets.[7,15] The coupling patterns of these diastereotopic methylene protons of the bis(menthyloxyacetyl) derivatives of the *trans*-tetrahydrodiols (Figure 3) were very similar to those of the bis(menthyloxyacetyl) derivatives of the corresponding non-fluorinated tetrahydro 9,10-diols of benzo[*a*]pyrene, whose absolute configurations have been established by chemical correlation[16] with other benzo[*a*]pyrene derivatives of known absolute configuration.[17] Thus, the (9*R*,10*S*)- and (9*S*,10*R*)-tetrahydroepoxides, precursors of the (9*R*,10*R*)- and (9*S*,10*S*)-tetrahydrodiols, respectively, were formed in a ratio of 32 to 68 (Scheme I). These results indicate that *N*-tosylimidazole has a slight preference for reaction with the C_9 hydroxyl group rather than with the more sterically hindered bay-region C_{10} hydroxyl group.

Table III. Rate Constants for the Solvolyses of 7,8-Diol 9,10-Epoxides and Tetrahydro-9,10-epoxides in 1:9 Dioxane–Water (Ionic Strength 0.1 M ($NaClO_4$), 25 °C)

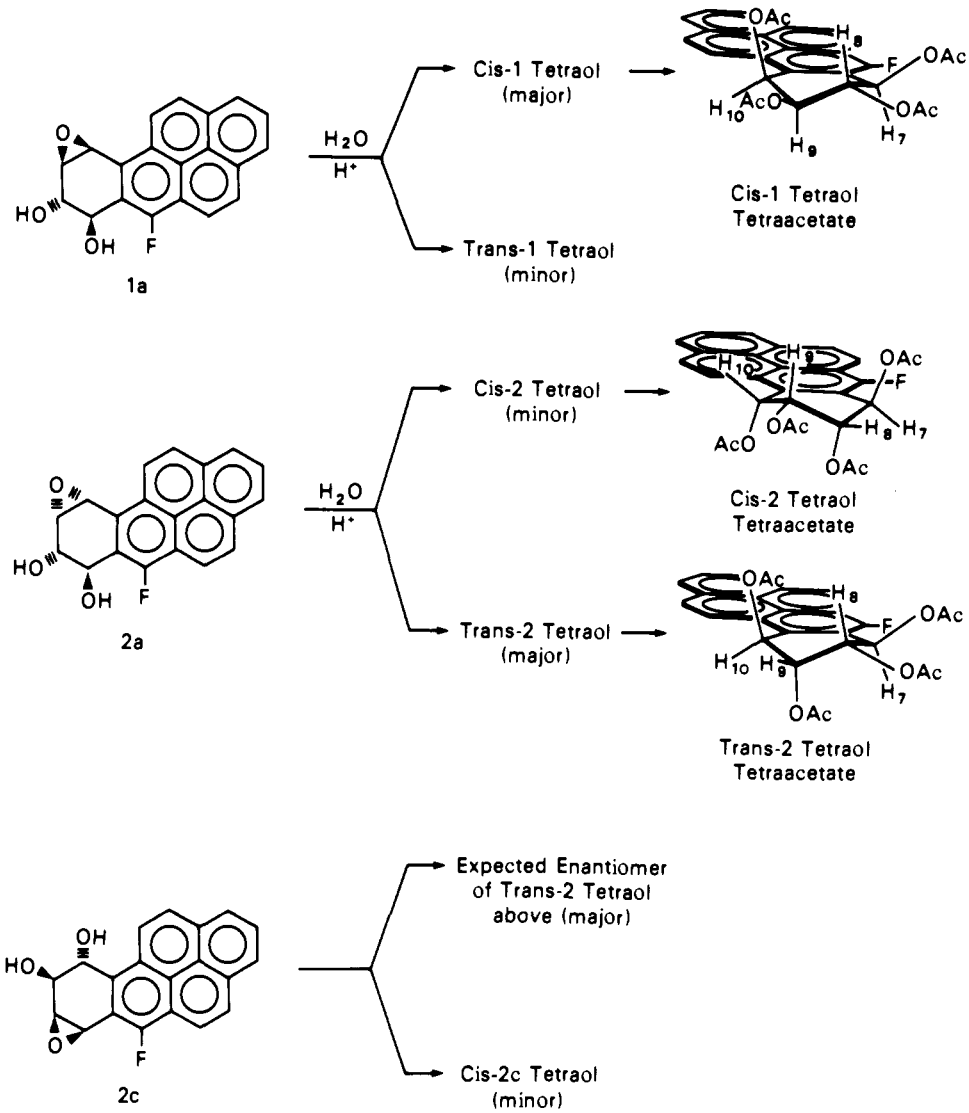
parent hydrocarbon[a] (compound)	k_H, M^{-1} s^{-1}	10^5k_0, s^{-1}
Isomer 1 Diol Epoxides		
BP (**1b**)	510	420
6-FBP (**1a**)	70	1.5
Isomer 2 Diol Epoxides		
BP (**2b**)	1400	13
6-FBP (**2a**)	200	45
Tetrahydroepoxides		
BP (**3b**)	19800	120
6-FBP (**3a**)	3000	11

[a] Data for the benzo[*a*]pyrene (BP) derivatives are from ref 18.

Diol Epoxide Hydrolysis Products. Preparative scale acidic hydrolyses of diol epoxides **1a** and **2a** were carried out to determine the relative stereochemistry of the tetraol products (Scheme II). After separation of the tetraols formed by cis and trans addition of water at the benzylic C_{10} position of diol epoxides **1a** and **2a**, each tetraol was acetylated. The ^{1}H NMR spectra of the tetraol tetraacetates are summarized in Table II. The major tetraol (~92%) derived from **1a** by acid-catalyzed hydrolysis was assigned the structure shown as *cis*-1 tetraol. The observed coupling constants for its tetraacetate are consistent with a predominant half-chair conformation and are in good agreement with the coupling constants for the corresponding *cis*-1 tetraol tetraacetate derived from the unfluorinated benzo[*a*]pyrene diol epoxide-1.[3a] The major (~80%) and the minor (~20%) tetraols obtained by acid-catalyzed hydrolysis of diol epoxide **2a** were assigned the structures shown as tetraols *trans*-2 and *cis*-2, respectively. The structure of the *trans*-2 tetraol was independently confirmed by comparison with the tetraol tetraacetates derived on hydrolysis of the "reverse" diol epoxide 6-FBP 9,10-diol 7,8-epoxide, **2c** (Scheme II), obtained by peroxyacid oxidation of 6-FBP 9,10-dihydrodiol, (−)-**5** (see Experimental Section). As expected, oxidation of this pseudoaxial dihydrodiol produced an equimolar mixture of the diastereomeric diol epoxides **1c** and **2c**. The observed coupling constants of both *trans*-2 and *cis*-2 tetraol tetraacetates are consistent with half-chair forms with the two acetate groups at C_9 and C_{10} pseudoaxial for the *trans*-2 derivative and the three acetate groups at C_7, C_8, and C_{10} pseudoaxial for the *cis*-2 derivative, respectively. The coupling constants, $J_{7,8}$, of *cis*-1 and *trans*-2 tetraol tetraacetates are slightly smaller than the corresponding $J_{7,8}$ values for their nonfluorinated counterparts and are consistent with some contribution of the corresponding

(15) Yagi, H.; Vyas, K. P.; Tada, M.; Thakker, D. R.; Jerina, D. M. *J. Org. Chem.* **1982**, *47*, 1110–1117. Yagi, H.; Thakker, D. R.; Ittah, Y.; Croisy-Delcey, M.; Jerina, D. M. *Tetrahedron Lett.* **1983**, *24*, 1349–1352.
(16) Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 4026–4027.
(17) Yagi, H.; Akagi, H.; Thakker, D. R.; Mah, H. D.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 2358–2359. Nakanishi, K.; Kasai, H.; Cho, H.; Harvey, R. G.; Jeffrey, A. M.; Jennette, K. W.; Weinstein, I. B. *Ibid.* **1977**, *99*, 258–260. Boyd, D. R.; Gadaginamath, G. S.; Kher, A.; Malone, J. F.; Yagi, H.; Jerina, D. M. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2112–2116. Boyd, D. R.; Gadaginamath, G. S.; Hamilton, R.; Yagi, H.; Jerina, D. M. *Tetrahedron Lett.* **1978**, 2487–2490.

Scheme II



half-boat form to the conformational equilibrium. However, this effect of the peri-fluorine at the C_6 position is not large enough to drive the conformational equilibrium completely toward the half-boat form. This unusual half-boat conformation was previously observed to predominate ($J_{diol} \sim 5$ Hz) in the case of the corresponding triphenylene and benzo[*e*]pyrene derivatives with two bay regions.[12a] Thus, it appears that the conformational effect of a peri-fluoro group in forcing an adjacent oxygenated substituent into a pseudoaxial orientation is somewhat less strong than that of a bay region.

Kinetics of Solvolysis. Like other diol epoxides and tetrahydroepoxides studied to date, compounds **1a–3a** undergo solvolysis in aqueous solution according to the rate law $k_{obsd} = k_H a_{H^+} + k_0$. Values of the rate constants measured in 1:9 dioxane–water, ionic strength 0.1, at 25 °C, in the presence of 10^{-3} M buffers as required for pH control, along with corresponding rate constants for the unsubstituted benzo[*a*]pyrene derivatives, are listed in Table III. The pH–rate profiles (Figure 4) resemble those previously observed for diol epoxides that have bay-region diol groups.[14] Notably, **1a** reacts ~30 times more slowly than **2a** under conditions of pH-independent solvolysis, whereas diastereomer-1 isomers of "normal" diol epoxides that lack unusual conformational features generally react from 3 to 30 times more rapidly than the corresponding diastereomer-2 isomers.[3c,18] This reversal of relative reactivities for diol epoxides with bay-region diol groups has been ascribed to alterations in their conformational equilibria.[14] Specifically, diastereomer-2 isomers with *pseudoaxial* hydroxyl groups have the benzylic C–O bond of the epoxide that is cleaved aligned with the π orbitals of the aromatic system, such that good orbital overlap is possible in the transition state for C–O cleavage, and acceleration of k_0 is observed for this conformation. Conversely, in diastereomer 1 isomers with a *strong preference* for *pseudoaxial* hydroxyl groups, this benzylic C–O bond of the epoxide is forced into a conformation (nonaligned with the π system) in which such orbital overlap is less favorable, leading to their decreased reactivity in the k_0 reaction (cf. ref 5).

To determine the effect of conformation on the solvolysis rates of the 6-FBP diol epoxides relative to their unsubstituted analogues, correction must be made for the electronic effect of the fluoro substituent that should inductively destabilize a cation at C_{10}. This effect may be estimated from comparison of the rates of solvolysis of the corresponding tetrahydroepoxides, whose conformation should be little influenced by fluorine substitution. The 6-fluoro substituent was found to decrease k_H by ~7-fold in the tetrahydroepoxide series. Thus, based on the known rates for the unfluorinated analogues (Table III) and consideration of the electronic substituent effect only, k_H was estimated to be 73 M^{-1} s^{-1} for **1a** and 200 M^{-1} s^{-1} for **2a**. The *experimental* values of 70 and 200 M^{-1} s^{-1}, respectively, show that there is *no conformational effect on* k_H; the rate differences between BP and 6-FBP diol epoxides in acid-catalyzed hydrolyses can be accounted for exclusively by an electronic substituent effect of fluorine. This observation is in accord with our previous findings that confor-

(18) Whalen, D. L.; Montemarano, J. A.; Thakker, D. R.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 5522–5524.

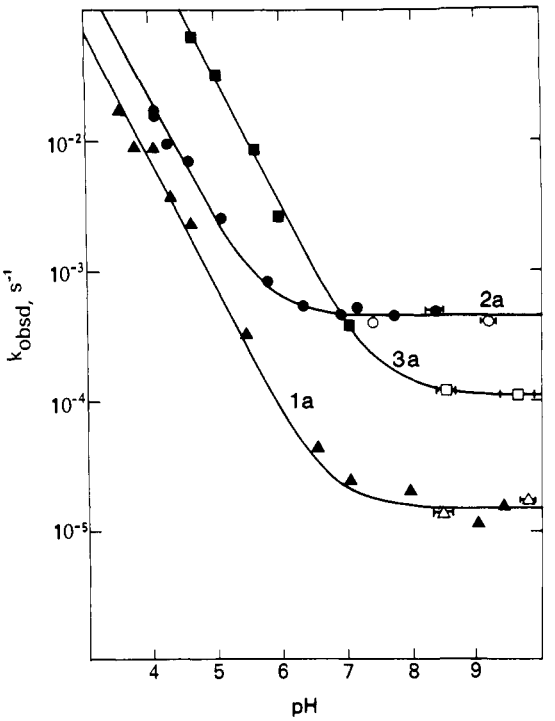


Figure 4. Effect of pH on the observed pseudo-first-order rate constants for solvolyses of **1a–3a** in 1:9 dioxane–water, at ionic strength 0.1 ($NaClO_4$). Solid symbols designate rate constants determined spectrophotometrically and open symbols designate rate constants determined by HPLC after trapping of unreacted epoxides.

Table IV. Products of the Hydronium Ion Catalyzed (k_H) and pH-Independent (k_0) Solvolyses of 7,8-Diol 9,10-Epoxides in 1:9 Dioxane–Water (Ionic Strength 0.1 M ($NaClO_4$), 25 °C)

parent hydrocarbon	pathway	% trans hydration	% cis hydration	% other or unrecovered products
		Isomer 1 Diol Epoxides		
BP[a]	k_H	11	89	
6-FBP	k_H	8	92	
BP[a]	k_0	9	84	~7
6-FBP	k_0	40	25	35[b]
		Isomer 2 Diol Epoxides		
BP[a]	k_H	92	8	
6-FBP	k_H	80	20	
BP[a]	k_0	46	54	
6-FBP	k_0	17	44	38[c]

[a] Reference 18. [b] Unrecovered product determined from the difference between the tetraols recovered upon acid hydrolysis and upon hydrolysis at pH 8.2–9.9 (average of three experiments). Although the keto diol could not be detected upon sodium borohydride trapping (see Experimental Section) it is presumably formed and lost since this product, but not the tetraols, was shown to be unstable under the reaction conditions. [c] Product identified as the keto diol on the basis of a trapping experiment with sodium borohydride (see Experimental Section); quantitation is based on peak areas of the borohydride reduction products.

mational effects on k_H for diol epoxides are small or negligible.[5,14] On the other hand, consideration of the substituent effect on k_0 (~11-fold expected decrease, based on the tetrahydroepoxides) gave estimates of k_0 for **1a** of 38 s^{-1} and for **2a** of 1.2 s^{-1}. The experimentally observed values of 1.5 s^{-1} for **1a** and 45 s^{-1} for **2a** correspond to essentially quantitative reversal of the expected relative reactivities based on an electronic effect alone. This observation can be explained on the basis of the *altered conformations* of **1a** and **2a** relative to the unsubstituted benzo[*a*]pyrene (BP) derivatives. In the case of isomer 1, the BP diol epoxide exists as an equilibrium mixture containing substantial amounts of both conformers 1′ and 1″ (cf. Figure 1). In the 6-fluoro derivative, as in diol epoxides with bay-region diol groups, the less reactive conformer 1′ predominates, resulting in a decrease in reactivity. In the case of isomer 2, the BP diol epoxide exists predominantly as the relatively unreactive nonaligned conformer 2′, whereas the 6-fluoro derivative, **2a**, exists as a mixture of approximately equal amounts of the two conformers 2′ and 2″ (Figure 2). Thus, a more favorable equilibrium for formation of the more reactive conformer results in the observed rate acceleration for **2a** relative to **2b**.

It is of interest to compare the rates for the k_0 reaction of epoxides derived from 6-FBP with those for the reaction of the corresponding benzo[*e*]pyrene and triphenylene derivatives.[14] In all three cases, NMR spectra indicate that the diol epoxide 1 diastereomers exist almost exclusively in the nonaligned conformation, 1′. The values of k_0 for hydrolysis of the tetrahydroepoxides derived from 6-FBP, benzo[*e*]pyrene, and triphenylene are 11×10^{-5}, 9.8×10^{-5}, and 13×10^{-5} s^{-1}, respectively. Thus, for diol epoxide 1, both electronic effects and conformation in the three series appear similar. Accordingly, in the isomer 1 series, values of k_0 for the three diol epoxides are 1.5×10^{-5}, 1.3×10^{-5}, and 0.53×10^{-5} s^{-1}, respectively. In the diol epoxide 2 series, values of k_0 for **2a**, benzo[*e*]pyrene diol epoxide 2, and triphenylene diol epoxide 2 are 45×10^{-5}, 2.0×10^{-5}, and 1.9×10^{-5} s^{-1}, respectively. The reasons for the markedly higher value of k_0 for **2a**, relative to diol epoxide 2 isomers with bay-region hydroxyl groups, are not clear. Similar values of k_0 for all three tetrahydroepoxides indicate similar electronic effects, and NMR spectra indicate that the reactive conformation, 2″, is less strongly favored for **2a** than it is for the diol epoxides with bay-region diol groups.

Products of Solvolysis. Some of the possible pathways for product formation from **1a** and **2a** are shown in Figures 1 and 2. Although, as previously noted, the negligible effect of conformation on the *rates* of the k_H reaction for 6-FBP diol epoxides was not unexpected, we anticipated that the unusual conformations of these diol epoxides might result in altered *product* distributions, relative to the BP derivatives, under conditions of acid-catalyzed hydrolysis. On the basis of our previous suggestion that the products of the k_H reaction are formed by preferential pseudoaxial attack of water upon a cation derived from the epoxide,[5] we predicted that **1a** should give substantially more trans hydration under acid conditions than the corresponding BP diol epoxide, since the following two factors could favor the cation (C^+1', Figure 1), which undergoes trans, pseudoaxial hydration, as the predominant reactive species. (i) If trapping of the cation by water is faster than conformational equilibration, as might be possible because of the destabilizing inductive effect of the 6-fluoro substituent, ring opening of the predominant conformer (1′) of **1a** should give this cation, C^+1', as the initially formed species that reacts with water. (ii) Even if conformational equilibration of the cation can occur prior to water attack, C^+1' was expected to be preferred over C^+1'', because of the interaction between the benzylic hydroxyl and the peri-fluoro group. Our prediction proved to be incorrect, as essentially the same low amount of trans hydration was observed for **1a** and for **1b**. This is in contrast to triphenylene diol epoxide 1 which gives 85% trans hydration in acid. We interpret this result as indicating (i) that some conformational equilibration of the cation takes place prior to water attack and (ii) that, contrary to expectation, cation C^+1'' is preferred over C^+1' for 6-FBP derivatives. This may be a consequence of the fact that a 6-fluoro substituent is less effective in forcing the axial orientation of the hydroxyl groups than is a second bay region, and in these cations an unfavorable 1,3-diaxial interaction between the hydroxyl groups at C_7 and C_9 is avoided by equilibration to C^+1''.

In the diol epoxide 2 series, trans hydration occurs to a slightly lesser extent with **2a** (80%) than with BP diol epoxide 2 (92%) but to a slightly greater extent than with triphenylene diol epoxide 2 (70%). This is in accordance with the idea that conformation C^+2' (Figure 2), which gives trans hydration, is least favorable when the hydroxyl groups are in a bay region, is somewhat more favorable in the presence of a peri-fluoro substituent, and is most strongly preferred for "normal" diol epoxides in the isomer 2 series, such as BP diol epoxide 2.

Detailed mechanistic interpretation of the observed products of the pH-independent (k_0) reaction is not feasible because of the numerous possible pathways for this process.[14] For tetraol formation, these possibilities include (i) S_N2 attack of water (possibly catalyzed by a second water molecule) to give trans ring opening, (ii) proton donation by water to the epoxide oxygen with ring opening, to generate an ion pair that collapses by cis attack of the hydroxide ion (derived from water) on the benzylic carbocation, and (iii) protonation by water with epoxide ring opening, followed by diffusional separation of the ion pair, to give the same free carbocation that is formed in the k_H process. In addition, mechanisms involving a zwitterionic carbocation/oxyanion intermediate can be written. For keto diol formation, it is likely that, regardless of whether the hydride shift occurs by a process concerted with ring opening or by a stepwise mechanism via a zwitterion, the aligned conformer should rearrange to keto diol more readily, since in this conformer the migrating hydrogen, H_9, occupies a more nearly pseudoaxial position. In the isomer 2 series, keto diol formation is unique to those molecules for which the aligned conformation, 2″, is significant.[14] Notably, a substantial amount of keto diol is formed from **2a**. The failure to recover 100% of the k_0 product from **1a** as tetraols suggests that the keto diol, which was unstable under conditions where the k_0 reaction predominates for **1a**, was also formed from this isomer, but was lost by decomposition. Thus, the k_0 reaction of **1a** may also proceed in part via the more reactive aligned conformer, 1″, or its corresponding zwitterion, despite the fact that this conformation is highly unfavorable at equilibrium in the reactant state.

Summary and Conclusions

The present study has shown that the presence of a 6-fluoro substituent markedly alters the conformational preferences of benzo[*a*]pyrene diol epoxides and their acetylated hydrolysis products. Since fluorine and hydrogen have similar steric requirements, this conformational effect results predominantly from an unfavorable electrostatic interaction between the fluorine substituent and the neighboring benzylic hydroxyl group, such that this hydroxyl group has a greater preference for a pseudoaxial orientation in the fluorinated, as opposed to the unfluorinated, BP derivatives. Chemical reactivity of the fluorinated diol epoxides in pH-independent solvolysis reactions (k_0) can be explained by a combination of inductive effects (which decrease k_0 for both isomer 1 and isomer 2 of the 6-FBP as compared to the BP diol epoxides) and conformational effects. The conformation in which the benzylic C–O bond that is cleaved in solvolysis is aligned with the π orbitals of the aromatic system is favored for diol epoxide 2 of 6-FBP relative to BP. This results in enhanced reactivity for the fluorinated diol epoxide. For isomer 1, this aligned conformation is highly unfavorable in the case of 6-FBP, and the resultant value of k_0 is much lower than would be expected on the basis of the inductive effect alone. These stereoelectronic effects on rates are negligible in hydronium ion catalyzed hydrolysis (k_H) reactions, although inductive effects on k_H are substantial. The presence of a 6-fluoro substituent appears to impose *a less severe constraint* upon the conformation of the hydroxyl groups of 6-FBP diol epoxides and related compounds than does location of these hydroxyl groups in a bay region, as reflected by the NMR spectra of the diol epoxides and their tetraol tetraacetates. Furthermore, the distribution of hydrolysis products from 6-FBP diol epoxide 1, unlike that from a diol epoxide with both the diol and the epoxide group in bay regions, supports the conclusion that a cation with pseudoequatorial hydroxyl groups may participate significantly in the reactions of the fluorinated diol epoxide.

Initiation-promotion experiments on mouse skin have shown that 6-FBP is far less tumorigenic than is BP.[9] This does not appear to be a consequence of altered metabolism since both hydrocarbons are converted to their (7*R*,8*S*)-diol (9*S*,10*R*)-epoxide 2 isomers to very similar extents by liver enzymes.[13] In the BP case, only the (+)-(7*R*,8*S*)-diol (9*S*,10*R*)-epoxide 2 isomer of the four metabolically possible 7,8-diol 9,10-epoxides has high tumorigenic activity.[1] The presence of a 6-fluoro substituent on this optically active isomer causes little change in size of the molecule and results in only a modest (3-fold) increase in reactivity in spontaneous solvolysis reactions. Thus, without changing other pertinent molecular characteristics, a conformational change from pseudoequatorial to pseudoaxial hydroxyl groups has been effected, so that the role of conformation in the expression of biological activity can be explored by comparison of the (7*R*,8*S*)-diol (9*S*,10*R*)-epoxide 2 isomers of 6-FBP and BP, **2a** and **2b**, respectively. Mutagenicity studies in Chinese hamster V79 cells, a rather good in vitro test system for predicting tumorigenic activity, have shown that **2a** has only 13% of the activity of **2b**.[13] Tumor studies done by intraperitoneal injection into Swiss–Webster newborn mice have shown that **1a** and **2a** are inactive, whereas the highly tumorigenic (+)-(7*R*,8*S*,9*S*,10*R*)-enantiomer of **2b** gave nearly two lung tumors per mouse at a 14-nmol dose.[19] Thus, pseudoaxial hydroxyl groups in bay-region diol epoxides appear to be a deterrent to the expression of tumorigenic activity.

Experimental Section

^{1}H NMR spectra were obtained with JEOL FX-100 and Nicolet 500 instruments. Chemical shift data are reported in parts per million (δ) downfield from internal tetramethylsilane with coupling constants (J) in hertz. Chemical ionization mass spectra ($NO-N_2$ or NH_3) were run on a Finnigan Model 1015D gas chromatograph mass spectrometer by the direct inlet mode, and only selected peaks are reported. Tetrahydrofuran (THF) was purified by distillation from $LiAlH_4$ just prior to use. Dioxane was purified by distillation from sodium. Me_2SO was purified by distillation from calcium hydride in vacuo. Enzymatically formed 7,8- and 9,10-dihydrodiols of 6-FBP were obtained as described.[13]

Epoxidation of (−)-*trans*-(7*R*,8*R*)-7,8-Dihydroxy-7,8-dihydro-6-fluorobenzo[*a*]pyrene [(−)-4]. The following procedure was carried out in the dark. To a cooled solution (−15 °C) of 9.6 mg of (−)-*trans*-7,8-dihydroxy-7,8-dihydro-6-fluoro-BP (metabolically obtained) in THF (0.5 mL) was added *m*-chloroperoxybenzoic acid (100 mg) under argon gas. The reaction mixture was gradually brought to room temperature and allowed to stand for 2 h, at which time its UV spectrum showed only the tetrahydro-BP chromophore. Cold ethyl acetate (100 mL) was added, and the resultant solution was washed with cold 1.5% NaOH (2 × 20 mL) and water (10 mL) and dried (K_2CO_3). Evaporation of the solvent gave 6 mg of mixed diol epoxide diastereomers which was subjected to HPLC on a Du Pont Zorbax SIL column (6.2 × 250 mm) eluted with hexane containing 30% dioxane (distilled just before use). The solvent reservoir was purged with argon during the course of separation. Evaporation of the combined less polar fractions (k' = 4.3) gave 2 mg of **(7*R*,8*S*,9*R*,10*S*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (1a)** as an amorphous solid: ^{1}H NMR (see Table I); mass spectrum ($NO-N_2$), m/z 320 (M^+); UV λ_{max} (ϵ) in THF, 345 (51 900), 329 (35 800), 315 (13 600), 279 nm (55 600), 268 (30 900), and 247 nm (95 100). Evaporation of the combined more polar fractions (k' = 4.9) gave 1.9 mg of **(7*R*,8*S*,9*S*,10*R*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (2a)** as an amorphous solid: ^{1}H NMR (see Table I); mass spectrum ($NO-N_2$), m/z 320 (M^+); UV λ_{max} (ϵ) in THF, 345 (51 900), 329 (36 000), 315 (13 700), 279 (55 600), 268 (31 000), and 247 nm (95 100). Both diol epoxides are very unstable and are best stored in anhydrous Me_2SO.

(−)-*trans*-(9*R*,10*R*)-9,10-Dihydroxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene [(−)-6]. A solution of 12.8 mg of (−)-*trans*-(9*R*,10*R*)-9,10-dihydroxy-9,10-dihydro-6-fluorobenzo[*a*]pyrene [(−)-**5**: metabolically obtained; $[\alpha]_D$ −214° (*c* 0.15, THF)] in ethyl acetate (7 mL) was hydrogenated at atmospheric pressure and room temperature in the presence of Pt (2.0 mg) for 5 min and then filtered to remove the catalyst. The filtrate was evaporated to leave a crystalline powder (13 mg): $[\alpha]_D$ −18° (*c* 0.35, THF); ^{1}H NMR (100 MHz, $CDCl_3$) δ 4.36 (m, 1 H, H_9), 5.48 (d, 1 H, H_{10}, $J_{9,10}$ = 4 Hz), 7.9–8.2 (m, 6 H, aromatic protons), and 8.42 (d, 1 H, H_{11}, $J_{11,12}$ = 10 Hz).

9,10-Epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (3a). To a mixture of sodium hydride (50%) dispersed in mineral oil (8 mg after washing with hexane) and THF (2 mL) was added a solution of the (−)-*trans*-9,10-tetrahydrodiol (−)-**6** (12.8 mg) in THF (3 mL). The mixture was stirred for 0.5 h, tosylimidazole (9.2 mg) was added, and the reaction mixture was stirred at room temperature for an additional 4 h. Cold ethyl acetate (150 mL) was added, and the organic phase was washed with ice-cold water (25 mL), filtered to remove an insoluble brownish precipitate, dried (Na_2SO_4), and evaporated to leave colorless crystalline material which was purified by HPLC on a silica column (Waters 5μ Radial Pak, 8 × 100 mm) eluted with 10% ether and 2.5%

(19) Levin, W.; Chang, R. L.; Wood, A. W.; Thakker, D. R.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1986**, *46*, 2257–2261.

triethylamine in cyclohexane. Evaporation of the appropriate fraction (k' = 3.0) gave 8 mg of the desired epoxide **3a**, mp 149–150 °C (hexane–ether): $[\alpha]_D$ +60° (*c* 0.15, THF); ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.6–3.5 (m, 4 H, 2 H_7 and 2 H_8), 3.96 (m, 1 H, H_9), 4.04 (q, 1 H, H_{10}, $J_{9,10}$ = 4.0, $J_{F6,10}$ = 2.0 Hz), 7.9–8.4 (m, 6 H, aromatic protons), and 8.65 (d, 1 H, H_{11}, $J_{11,12}$ = 10 Hz); mass spectrum (NH_3), *m/z* 306 (M + NH_4^+), 289 (M + 1)$^+$, and 288 (M + NH_4^+ – H_2O). This compound proved to be a 68:32 mixture of the (9*S*,10*R*) and (9*R*,10*S*) enantiomers, respectively (see Results and Discussion).

Acid-Catalyzed Hydrolysis of 3a. The 9,10-epoxide **3a** (1 mg) was dissolved in a mixture of THF (3 mL) and water (10 mL), which was adjusted to pH 2 with $HClO_4$ and which contained 0.1 M $NaClO_4$. After 2 h at 25 °C, the solution was neutralized with $NaHCO_3$ and evaporated to remove THF. Ethyl acetate (20 mL) was added, and the organic phase was washed with water (10 mL), dried (Na_2SO_4), and evaporated. Products were separated by HPLC on a Du Pont Zorbax ODS column (9.5 × 250 mm) eluted with 30% water in methanol at a flow rate of 2.4 mL/min. Evaporation of the early eluting fraction (retention time 7.8 min) afforded 0.3 mg of ***trans*-9,10-dihydroxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (6)** whose retention time on HPLC and ^{1}H NMR spectrum were identical with authentic material obtained by reduction of (–)-**5** above. Evaporation of the late eluting fraction (retention time 11.7 min) afforded 0.6 mg of ***cis*-9,10-dihydroxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene**: ^{1}H NMR (100 MHz, $CDCl_3$–CD_3OD) δ 4.02 (sextet, 1 H, H_9, $J_{9ax,8ax}$ = 12, $J_{9ax,8eq}$ = $J_{9ax,10eq}$ = 3.8 Hz), 5.66 (d, 1 H, H_{10}, $J_{9,10}$ = 3.8 Hz), 7.9–8.4 (m, 6 H, aromatic protons), and 8.56 (d, 1 H, H_{11}, $J_{11,12}$ = 10 Hz). The ratio of trans to cis diol was 34:66 (detected by UV at 280 nm).

Diastereoisomeric Bis(menthyloxyacetate) (MOA) Esters of *trans*-9,10-Dihydroxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene. To a solution of the *trans*-tetrahydrodiol **6** (0.3 mg, obtained above from acid-catalyzed hydrolysis of the tetrahydroepoxide **3a**) in pyridine (0.5 mL) was added the acid chloride of (–)-menthyloxyacetic acid (10 mg), and the reaction mixture was allowed to stand at room temperature overnight. Pyridine was evaporated under reduced pressure, benzene (15 mL) was added, and the organic phase was subjected to standard workup and evaporated. The diastereomers in the residue were separated by HPLC on a Du Pont Zorbax SIL column (6.2 × 250 mm) eluted with 10% ether in cyclohexane at a flow rate of 2.2 mL/min. Evaporation of the combined less polar fractions (32%, k' = 1.9) afforded the bis-MOA ester of the ***trans*-(9*R*,10*R*)-tetrahydro-9,10-diol (–)-6**: ^{1}H NMR (100 MHz, benzene-d_6) signals centered at 3.64 and 3.88 (dd, 2 H, OCH_2CO_2, J_{gem} = 16 Hz), 3.96 (s, 2 H, OCH_2CO_2), 5.75 (m, 1 H, H_9), and 7.28 (d, 1 H, H_{10}, $J_{9,10}$ = 4.0 Hz). Partial ^{1}H NMR spectra are shown in Figure 3. This compound is identical with the bis-MOA ester of the diol obtained on reduction of the metabolically formed 9,10-dihydrodiol of 6-FBP (Scheme I). Evaporation of the combined more polar fractions (68%, k' = 2.6) afforded the bis-MOA ester of the ***trans*-(9*S*,10*S*)-tetrahydro-9,10-diol (+)-6**: ^{1}H NMR (100 MHz, benzene-d_6) signals centered at 3.64 and 3.86 (dd, 2 H, OCH_2CO_2, J_{gem} = 16 Hz), centered at 3.94 and 4.04 (dd, 2 H, OCH_2CO_2, J_{gem} = 16 Hz), 5.75 (m, 1 H, H_9), and 7.28 (d, 1 H, H_{10}, $J_{9,10}$ = 4.0 Hz).

Hydrolysis of Diol Epoxides 1a and 2a to Tetraols. Samples of diol epoxides **1a** and **2a** (~1 mg) were dissolved separately in 10 mL of 1:9 dioxane–water (0.1 M $NaClO_4$) which was adjusted to pH 2 with $HClO_4$. After 2 h at room temperature (>10 × $t_{1/2}$), the dioxane was evaporated, and the product tetraols were extracted into ethyl acetate prior to HPLC on a Du Pont Zorbax ODS column (21.2 × 250 mm). *trans*-1 and *cis*-1 tetraols were eluted with 45% CH_3CN in water at a flow rate of 20 mL/min: *cis*-1 tetraol (4.0 min), *trans*-1 tetraol (5.3 min). *trans*-2 and *cis*-2 tetraols were eluted with 70% MeOH in water at a flow rate of 15 mL/min: *trans*-2 tetraol (7.1 min), *cis*-2 tetraol (11.2 min). Each tetraol was acetylated in an excess of pyridine and acetic anhydride overnight. Usual workup afforded the corresponding tetraol tetraacetates which were purified by HPLC on a Waters Radial Pak silica column (5 μm, 8 × 100 mm) eluted with 10% ethyl acetate in methylene chloride prior to the measurement of their ^{1}H NMR spectra (Table II). Mass spectra (NO–N_2) of all of the above tetraol tetraacetates showed the expected M^+ at *m/z* 506.

Epoxidation of (–)-*trans*-(9*R*,10*R*)-9,10-Dihydroxy-9,10-dihydro-6-fluorobenzo[*a*]pyrene [(–)-5]. The epoxidation of metabolically formed (–)-**5** (5.8 mg) with *m*-chloroperoxybenzoic acid (58 mg) in THF (1 mL) was carried out as described above for the 7,8-dihydrodiol. The crude product was separated by HPLC on a Du Pont Zorbax SIL column (6.2 × 250 mm) eluted with 40% dioxane in hexane. Evaporation of the combined less polar fractions (k' = 4.0) gave the diol epoxide 1 diastereomer **(7*S*,8*R*,9*S*,10*R*)-9,10-dihydroxy-7,8-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (1c)** (2 mg) as an amorphous solid. Evaporation of the combined more polar fractions (k' = 5.5) gave the diol epoxide 2 diastereomer **(7*R*,8*S*,9*S*,10*R*)-9,10-dihydroxy-7,8-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene (2c)** (2 mg) as a colorless solid. Both diol epoxides gave mass spectra (NO–N_2) with *m/z* 320 (M^+). ^{1}H NMR data are in Table I. Particularly noteworthy is $^4J_{8,10}$ = 2.5 Hz for diol epoxide **1c**. As noted in the Results and Discussion, such a four-bond coupling is only possible for a diol epoxide 1 diastereomer.

Hydrolysis of Diol Epoxide 2c to Tetraols. Acid-catalyzed hydrolysis of **2c** (1 mg) was carried out in 1:9 dioxane–water at pH 2 as described above for **1a**. The hydrolysate was separated by HPLC on a Du Pont Zorbax ODS column (4.2 × 250 mm) eluted with 60% MeOH in water at a flow rate of 1.2 mL/min to give a major (85%, 5.5 min) and a minor (15%, 7.1 mm) tetraol. The major tetraol (*trans*-2 tetraol, Scheme II) was collected and subjected to acetylation in an excess of pyridine and acetic anhydride as described above. The ^{1}H NMR spectrum of this tetraacetate was superimposable upon that of the tetraacetate of the *trans*-2 tetraol derived from diol epoxide **2a**.

Kinetics of Diol Epoxide Hydrolysis. Rates of reaction were measured at 25 °C in 1:9 dioxane–water containing 0.1 M $NaClO_4$ and 10^{-3} M buffers (sodium formate, sodium acetate, tris(hydroxymethyl)aminomethane, BES (*N*,*N*-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid), CHES (2-[cyclohexylamino]ethanesulfonic acid), and MES (2-[*N*-morpholino]ethanesulfonic acid). Pseudo-first-order reactions were followed with a Cary 219 spectrophotometer at the following wavelengths (nm): **1a**, 249 or 277 (pH <6), 385 (pH >6); **2a**, 249 or 277; **3a**, 277; **3b**, 347 (pH <7), 350 (pH >7). The wavelengths used at the higher pH values were selected to minimize endpoint drift that presumably resulted from secondary reactions of an unstable ketonic product from the pH-independent reactions.

Reactions of **1a**, **2a**, and **3a** at pH >7 were also followed by HPLC after trapping of the unreacted epoxides as the mercaptoethanol adducts. Chromatographic systems used were as follows: for **1a** and **2a** (system A), a Du Pont Zorbax ODS column (4.6 × 250 mm) eluted with a linear gradient of 50–75% methanol in water at a flow rate of 0.8 mL/min and a rate of gradient change of 1% per min; for **3a** (system B), a Waters Associates μBondapak C18 column (3.9 × 300 mm) eluted with a linear gradient of 60–85% methanol in water at a flow rate of 1.2 mL/min and a rate of gradient change of 1% per min. In typical trapping experiments, 0.3-mL aliquots of a reaction mixture containing ~7–8 × 10^{-6} M diol epoxide and 7 × 10^{-5} M acetophenone internal standard, or ~2 × 10^{-6} M tetrahydroepoxide and 2.5 × 10^{-5} M *n*-butyrophenone internal standard, were quenched at various times with 0.06 mL of 2 M mercaptoethanol, 20% as the sodium salt. Trapped epoxide (as the thioether) was quantified by integration of the peak area (277 nm) corresponding to this product, relative to the internal standard. In system A, the tetraols formed by hydrolysis of **1a** elute as a poorly resolved pair of peaks at ~20–21 min, and the thioether from **1a** elutes at 23.4 min, whereas the *trans*-2 tetraol, thioether, and *cis*-2 tetraol from **2a** elute at 15.6, 18.4, and 20 min, respectively. In system B, the thioether derivative of **3a** elutes at 15.3 min. Other observed products of the pH-independent reaction of **3a** (with elution times of 9.0, 13.9, and 19.4 min) presumably correspond to the *trans*- and *cis*-diols and probably to a ketone or its degradation product. For diol epoxides **1a** and **2a**, rate constants determined from the time dependence of the decrease in the thioether peak area were in good agreement with rate constants determined spectrophotometrically. For **3a**, spectrophotometric kinetic measurements were not possible above pH 7 because of unstable end points presumably resulting from product degradation.

Analysis of Products Formed under Kinetic Conditions. Because of the poor separation of the *trans*-1 and *cis*-1 tetraols from **1a** in chromatographic systems with methanol–water mixtures as the eluent, the relative amounts of these tetraol products formed under kinetic conditions were determined by HPLC on the Du Pont Zorbax ODS column eluted with a linear gradient of 25–50% acetonitrile in water at a flow rate of 0.8 mL/min. In this system the *cis*-1 tetraol elutes at 18.7 min and the *trans*-1 tetraol at 22.6 min. The recovery of tetraols from the k_0 reaction of **1a** and **2a** was determined from the difference in the peak areas (relative to internal standard) corresponding to *total tetraols* (determined in chromatographic system A) that are formed from identical samples under pH-independent solvolysis conditions and in 10^{-3} M perchloric acid. Recovery of 100% of the diol epoxides as tetraols under acid hydrolysis conditions was assumed. Recoveries of tetraols under k_0 conditions were ~65% and 60% from **1a** and **2a**, respectively. Although the keto diol formed from the k_0 reaction of **2a** was generally not detected upon direct chromatography of reaction mixtures after completion of reaction or thiol trapping of unreacted epoxide, due to the instability and/or poor chromatographic characteristics of this ketone, the formation of this product was demonstrated as follows. Diol epoxide **2a** was allowed to react in BES buffer, pH 7.1, for 2.5 h (5.8 × $t_{1/2}$), acidified to pH 2.8 to destroy any unreacted diol epoxide, readjusted to pH ~8, and reduced with excess sodium borohydride for 15 min. After extraction of the products into ethyl acetate, chromatography in system A showed (in addition to

the *trans*-2 and *cis*-2 tetraols (rt 16.2 and 20.6 min, respectively)) the presence of two new peaks (minor, rt 22.2 min; and major, rt 27.7 min). These peaks, which were ascribed to the two diastereomeric triols formed upon reduction of a keto diol, accounted for ~38% of the total products derived from **2a**. The larger of these two peaks gave an ultraviolet spectrum corresponding to a 7,8,9,10-tetrahydro-BP chromophore. In a similar experiment, **1a** was allowed to react for 26 h ($\sim 2 \times t_{1/2}$) at pH 8.2, acidified, and then treated with excess sodium borohydride. Chromatography of the resultant mixture indicated that no triols were present. Under similar conditions (overnight incubation at pH 7.9), the keto diol formed from **2a** was completely lost, as demonstrated by the inability to detect triols by borohydride trapping subsequent to this incubation.

Charge Separation in Carotenoporphyrin-Quinone Triads: Synthetic, Conformational, and Fluorescence Lifetime Studies

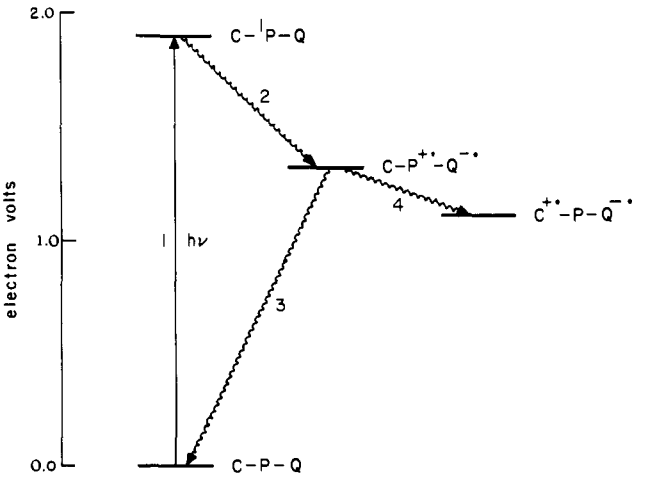
Devens Gust,*†,# Thomas A. Moore,*† Paul A. Liddell,† Gregory A. Nemeth,† Lewis R. Makings,† Ana L. Moore,† Donna Barrett,† Peter J. Pessiki,† René V. Bensasson,# Michel Rougée,# Claude Chachaty,‡ F. C. De Schryver,⊥ M. Van der Auweraer,⊥ Alfred R. Holzwarth,§ and John S. Connolly±

Contribution from the Department of Chemistry, Arizona State University, Tempe, Arizona 85287, the Laboratoire de Biophysique, ERA 951 du CNRS, Muséum National d'Histoire Naturelle, 75005 Paris, France, the Département de Physico-Chimie, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex, France, the Afdeling Organische Scheikunde, Katholieke Universiteit Leuven, Celestijnenlaan 200F, Leuven, Belgium, the Max Planck Institute fur Strahlenchemie, Mulheim, West Germany, and the Photoconversion Research Branch, Solar Energy Research Institute, Golden, Colorado 80401. *Received February 25, 1986*

Abstract: Carotenoid-porphyrin-quinone triad molecules undergo a photodriven two-step electron-transfer reaction which results in the generation of a high-energy charge-separated state with a lifetime on the microsecond time scale at ambient temperatures in fluid solution. These systems mimic the initial charge separation steps of photosynthesis. A series of these tripartite molecules which differ systematically in the nature of the linkages joining the porphyrin to the quinone and carotenoid moieties has been synthesized in order to investigate the effect of structure on the yield and lifetime of the charge-separated state. The time-averaged solution conformations of these molecules have been determined from porphyrin ring current induced shifts in the ^{1}H NMR resonances of the carotenoid and quinone moieties. Studies of the triads and related molecules in dichloromethane solution using time-correlated single photon counting fluorescence lifetime techniques have yielded the rate constant for the first of the photoinitiated electron-transfer steps as a function of the linkage joining the porphyrin and the quinone. The rate constants range from 1.5×10^8 to 9.7×10^9 s^{-1}. For most members of the series, the results are consistent with an exponential dependence of the electron-transfer rate on the experimentally determined donor-acceptor separation, with the exponential factor $\alpha = 0.6$ Å^{-1}.

Molecular triads, consisting of porphyrins covalently linked to both carotenoid polyenes and quinones, and related systems have been developed as models for photosynthetic charge separation, singlet energy transfer (antenna function), and triplet energy transfer (photoprotection from singlet oxygen via carotenoid quenching of the chlorophyll triplet state).[1-11] With respect to charge separation, triad molecules such as **1**, in common with porphyrin-quinone dyad molecules,[12] carry out photodriven electron transfer in good yield. In addition, the triads produce energetic charge-separated states with lifetimes on the microsecond time scale.[6-8,10,11] The key to long lifetimes in these systems is a biomimetic two-step intramolecular electron transfer (see Scheme I). Excitation of the porphyrin moiety (step 1) yields the porphyrin first excited singlet state C-^{1}P-Q, which donates an electron to the quinone to produce an initial charge-separated state C-P$^{\cdot+}$-Q$^{\cdot-}$ (step 2). This state has two possible pathways for decay. Charge recombination (step 3) is a facile reaction which yields the ground state. Such back-electron-transfer reactions are to be avoided in photosynthesis or other energy conserving systems because they degrade the chemical potential stored in the charge-separated state to heat. In the triads, a second electron-transfer reaction (step 4) competes with step 3 to yield a final charge-separated state C$^{\cdot+}$-P-Q$^{\cdot-}$. This state lives from hundreds

Scheme I



† Arizona State University.
# Muséum National d'Histoire Naturelle.
‡ Département de Physico-Chimie, CEN Saclay.
⊥ Katholieke Universiteit Leuven.
§ Max Planck Institute.
± Solar Energy Research Institute.

(1) Dirks, G.; Moore, A. L.; Moore, T. A.; Gust, D. *Photochem. Photobiol.* **1980**, *32*, 277-280.
(2) Moore, A. L.; Dirks, G.; Gust, D.; Moore, T. A. *Photochem. Photobiol.* **1980**, *32*, 691-695.

0002-7863/87/1509-0846$01.50/0