1987, *26*, 1026.
7. Efimov, O. N.; Strelets *Coord. Chem. Rev.* **1990**, *99*, 15
8. Ashmawy, F. M.; Issa, R. M.; Amer, S. A.; McAuliffe, C. A.; Parish, R. V. *J. Chem. Soc., Dalton Trans.* **1986**, 421.
9. McAuliffe, C. A.; Parish, R. V.; Ashmawy, F. M.; Issa, R. M.; Amer, S. A. *J. Chem. Soc., Dalton Trans.* **1987**, 2009.
10. Gosden, C.; Kerr, J. B.; Pletcher, D.; Rosas, R. *J. Electroanal. Chem.* **1981**, *117*, 101.
11. Isse, A. A.; Gennaro, A.; Vianello, E. *Electrochim. Acta* **1992**, *37*, 113.
12. Smith, W. E.; El-Shahawl, M. S. *Analyst* **1994**, *119*, 327.
13. Kotočová, A.; Šima, J.; Valigura, D.; Fodran, P. *Inorg. Chim. Acta* **1987**, *128*, 11.
14. Kotočová, A.; Valigura, D.; Šima, J. *J. Coord. Chem.* **1991**, *24*, 363.
15. Kotočová, A.; Šima, J. *Monmatsh. Chem.* **1994**, *125*, 491.
16. Kotočová, A.; Šima, J. *Chem. Papers* **1994**, *48*, 175.
17. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; Pergamon Press: Oxford, U. K., 1988.
18. Geary, W. J. *Coor. Chem. Rev.* **1971**, *7*, 81.
19. Boucher, L. J. *Inorg. Chem.* **1976**, *15*, 1334.
20. Ma, Y.-X.; Lu, Z.-L.; Song, Q.-B.; WU, X.-L. *J. Coor. Chem.* **1994**, 353.
21. McAuliffe, C. A.; McCullough, F. P.; Parrott, M. J.; Arlyn Rice, C.; Sayle, B. J.; Levason, W. *J. Chem. Soc. Dalton Trans.* **1977**, 1762.
22. Ueno, K.; Martell, A. E. *J. Phys. Chem.* **1956**, *27*, 2544.
23. Nakamoto, K.; Martell, A. E. *J. Chem. Phys.* **1960**, *32*, 588.
24. Sacconi, L.; Bertini, I. *J. Am. Chem. Soc.* **1966**, *88*, 5180.
25. Sarama, B. D.; Ray, K. R.; Sievers, R. E.; Bailar, J. *J. Am. Chem. Soc.* **1964**, *86*, 14.
26. Coleman, W. M.; Goehring, R. R.; Taylor, L. T.; Mason, J. G.; Boggess, R. K. *J. Am. Chem. Soc.* **1979**, *101*, 2311.
27. Bard, A. J.; Faulkner, L. R. *Electrochemical Methodes*; Chap. 5-6, John Wiley & Sons: New York, 1980.

Product Studies by HPLC on the Hydrolysis of the *anti*- and *syn*-Tetrahydrodiol Epoxides and the 1,2-Tetrahydro Epoxide of Naphthalene

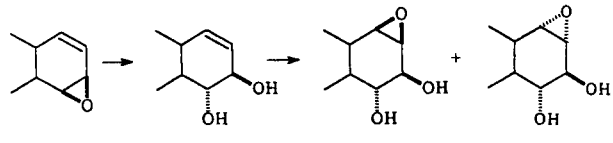
Yong Tae Lee* and Jed F. Fisher†

Department of Biochemistry, Yeungnam University, Kyongsan 712-749, Korea
†Discovery Chemistry Research, Pharmacia & Upjohn, Kalamazoo, Michigan 49007-4940, U. S. A.
Received May 16, 1997

The arene epoxides from naphthalene, 1β,2α-dihydroxy-3α,4α-epoxy- (**1**) and 1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydronaphthalene (**2**) (*anti*- and *syn*-diol epoxide), 1,2-epoxy-1,2,3,4-tetrahydronaphthalene (**3**), and 1,2-epoxy-1,2-dihydronaphthalene (**4**), are model componds of the ultimate carcinogenic metabolites of polycyclic aromatic hydrocarbons, ubiquitous environmental pollutants which may be causal in several human cancers. The product distribution in the hydrolysis of **1-4** have been studied by HPLC analysis of reaction mixtures. The yields of the trans product from the hydronium-ion-catalyzed and pH-independent hydrolysis in 9 : 1 (v/v) 20 mM buffer-dioxane at 25 °C, respectively, were; **1**: 98, 100; **2**: 74, 87, **3**: 95, 97, **4**:100, 100. The results were rationalized by conformational equilibria of the epoxides and the carbocationic and zwitterionic intermediates from the epoxides.

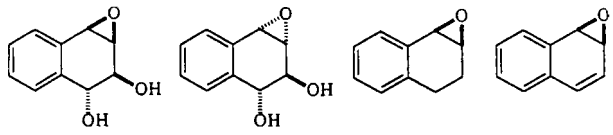
Introduction

Polycyclic aromatic hydrocarbons (PAH's) are widespread environmental pollutants which are considered to be an important class of mutagenic and carcinogenic compounds. They are metabolized in part by the cytochrome P-450 monooxygenases to arene epoxides followed by hydrolysis to the *trans*-diols and further oxygenation at the adjacent double bond to form diol epoxides.[1-3] These diol epoxides may express their electrophilic reactivity by initiating the carcinogenic process through DNA alkylation.[4] In an effort to understand the mechanism of the initiation of the PAH carcinogenesis, *in vitro* electrophilic reactivities of several PAH diol epoxides have been studied in detail.[5,6]



The following epoxides, **1-4** are model compounds of the metabolites of carcinogenic PAH's.

To date, kinetic studies on the hydrolysis of these epoxides have been undertaken by Bruice[7,8] and by the author,[9] but no product studies on their hydrolysis have been carried



1 2 3 4

out probably due to difficulty in chromatographic separation of the isomeric products. This paper reports the results of the product studies on the hydrolysis of epoxides **1-4**. Jerina *et al.*[10,11] have shown that conformational effects are important in determining the rates and products of hydrolysis of PAH diol epoxides and have proposed mechanisms for these effects. Since **1-4** have no unusual conformational features such as existence of bay region[12] in the molecule, they can be used as important reference compounds in rationalizing the proposed mechanisms.

Experimental

General. ^{1}H NMR spectra were obtained on a Nicolet NT-300 WB or IBM NR-300 AF NMR spectrometer. Chemical shifts (δ) were determined using TMS as an internal standard. UV-Vis spectra were recorded on a Varian Cary 219 spectrophotometer. A Fisher Acumet 815 MP pH meter was utilized for pH measurement. Liquid chromatography was performed on a Beckman 110 A dual pump system and a Hitachi 100-10 UV-Vis spectrophotometer. Epoxides **1-4** were synthesized by published procedures.[13] All buffers were made with water passed through a Barnstead NANO-pure water purification system. Dioxane, a cosolvent for the reaction and a component of the HPLC mobile phase, was purified by distilling over sodium.

Hydrolysis of the Epoxides. The hydrolysis reactions of the epoxides (1 mM) were run in 9 : 1 (v/v) 20 mM buffer-dioxane at 25 °C. The reactions were run at two pH's, one in the hydronium-ion-catalyzed (k_H) and the other in the pH-independent (k_0) region. The buffers and pH values (buffer-dioxane) employed were; for k_H reactions, acetate (5.11), formate (4.23), cacodylate (6.58) and cacodylate (6.20) for **1, 2, 3**, and **4**, respectively, and for k_0 reactions, Tris (7.93) for all epoxides.

Chromatography. To analyze the reaction mixtures by HPLC, samples were eluted isocratically on a Rainin Microsorb C-18 column (5 μm, 4.6 mm × 25 cm) at 0.8 mL min^{-1}. Detection was made spectrophotometrically at 210 nm. The mobile phase used were methanol-dioxane-water (34 : 1 : 65) for **1** and **2**, or acetonitrile-dioxane-0.01% Na_2HPO_4 or acetonitrile-dioxane-water (44 : 1 : 55) for **3** and **4**, respectively. The product distribution in each reaction after complete epoxide disappearance was analyzed by HPLC using mobile phases with much higher water content. The hydrolysis products were isolated by preparative HPLC using a DuPont Zorbax C-18 column (10 μm, 2.1 × 25 cm). A sample solution of 1.7 mL was injected and eluted at a flow rate of 4.5 mL min^{-1}.

Products from Epoxide 1. Reaction mixtures after completion of hydrolysis were combined and the major product (**5**) was isolated by preparative HPLC using 20 : 80 CH_3CN-H_2O as the mobile phase (t_R 17 min). The minor product (**6**) could not be separated from the major product by preparative HPLC. Analytical HPLC, however, allowed its isolation in a quantity sufficient only for obtaining UV-Vis spectrum.

1α,2β,3β,4α-Tetrahydroxy-1,2,3,4-tetrahydronaphthalene (5). UV (5% MeOH-H_2O): λ_{max} 210 nm. ^{1}H NMR (300 MHz, CD_3CN): δ 7.45-7.40 (m, 2H, H-5 and H-8), 7.34-7.31 (m, 2H, H-6 and H-7, symmetrical to δ 7.45-7.40 multiplet), 4.62 (d, 2H, H-1 and H-4, $J_{1,2}$=5.3 Hz), 3.99 (d, 2H, H-2 and H-3, symmetrical to δ 4.62 doublet).

1α,2α,3α,4β-Tetrahydroxy-1,2,3,4-tetrahydronaphthalene (6). UV (5% MeOH-H_2O): λ_{max} 211 nm.

Products from Epoxide 2. Both the *trans* (**7**) and *cis* (**8**) product were isolated by preparative HPLC using 30 : 70 MeOH-H_2O as the mobile phase. The retention times were 17.3 and 19.4 min, respectively.

1α,2β,3α,4β-Tetrahydroxy-1,2,3,4-tetrahydronaphthalene (7). UV (30% MeOH-H_2O): λ_{max} 210(100) & 260(3) nm. ^{1}H NMR (300 MHz, CD_3CN): δ 7.46 (q, 2H, H-5 and H-8), 7.28 (q, 2H, H-6 & H-7, symmetrical to δ 7.46 quartet), 4.50 (q, 2H, H-1 & H-4, J=5.7 & 2.5 Hz), 3.47 (q, 2H, H-2 & H-3, symmetrical to δ 4.50 quartet).

1α,2α,3β,4α-Tetrahydroxy-1,2,3,4-tetrahydronaphthalene (8). UV (30% MeOH-H_2O): λ_{max} 210(100) & 260(3) nm. ^{1}H NMR (300 MHz, CD_3CN): δ 7.51-7.29 (m, 4H, aryl), 4.71 (d, 1H, J=4.9 Hz), 4.38 (d, 1H, J=8.2 Hz).

Products from Epoxide 3. The major product (**9**) was isolated by preparative HPLC using 30 : 70 CH_3CN-H_2O as the mobile phase (t_R 18 min). The minor product was identified as *cis* diol (**10**) by coeluting the reaction mixture with the *cis* diol which was synthesized by hydroxylation of 1,2-dihydronaphthalene with OsO_4.

***trans*-1,2-Dihydroxy-1,2,3,4-tetrahydronaphthalene (9).** ^{1}H NMR (300 MHz, CD_3CN): δ 7.46-7.05 (m, 4H, aryl), 4.38 (d, 1H, H-1, $J_{1,2}$=7.0 Hz), 3.69 (ddd, 1 H, H-2, $J_{2,3(cis)}$=3.4, $J_{2,3(trans)}$=10.1 Hz), 2.84 (m, 1H, H-4), 1.97 (m, 1H, H-3), 1.75 (m, 1H, H-3).

***cis*-1,2-Dihydroxy-1,2,3,4-tetrahydronaphthalene (10).** ^{1}H NMR (300 MHz, CD_3CN): δ 7.36-7.06 (m, 4H, aryl), 4.55 (d, 1H, H-1, $J_{1,2}$=2.5 Hz), 3.89 (ddd, 1H, H-2, $J_{2,3(cis)}$=3.4, $J_{2,3(trans)}$=9.7 Hz), 2.91 (m, 1H, H-4), 2.88 (ddd, 1H, H-4', $J_{3,4}$=8.8, 6.3, 5.8, 5.8 Hz, $J_{4,4'}$=17.1 Hz), 1.94 (m, 1H, H-3), 1.80 (m, 1H, H-3').

Results and Discussion

The pH *vs.* rate profile for the epoxide hydrolysis follows the rate law:

$$k_{obsd}=k_0+k_H[H_3O^+]+k_{OH}[OH^-]$$

where k_0 is the pH-independent, and k_H and k_{OH} are the hydronium-ion-catalyzed and hydroxide-ion-catalyzed rate constant, respectively.[14] Thus the mechanism of the hydrolysis is changed as the pH of the reaction medium is varied, and the reactions in this study were run at two pH's, one in the k_H and the other in the k_0 region. The k_{OH} process contributes only at very high pH and is usually not of physiological relevance.

The analytical HPLC chromatograms from the k_H and k_0 reaction of epoxide 1 are presented in Figure 1(a) and 1(b), respectively. When the reaction mixtures from the k_H reac-

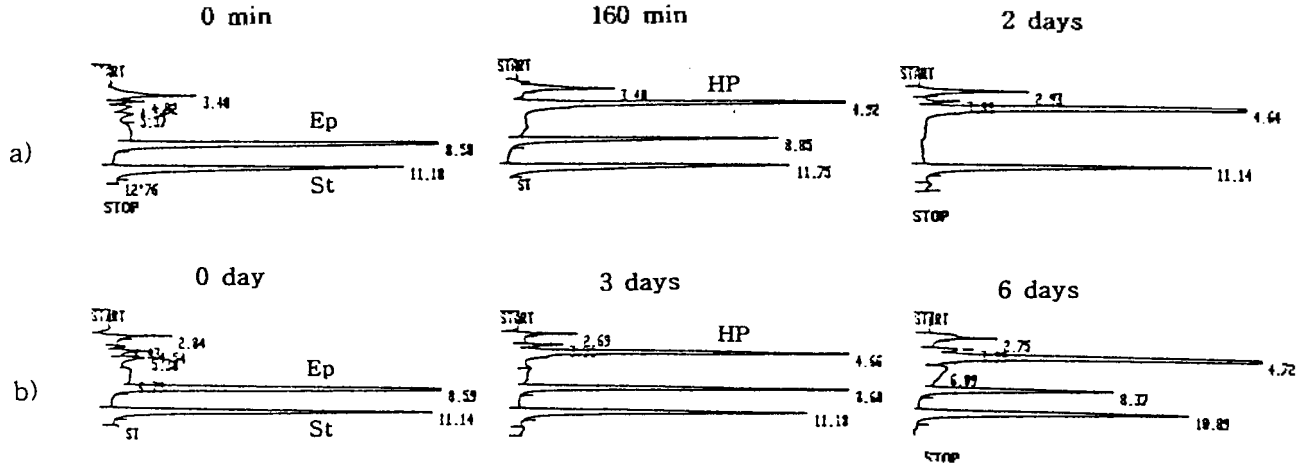


Figure 1. Time course by HPLC monitoring of the hydronium-ion-catalyzed (a) and pH-independent (b) hydrolysis of epoxide **1**. Reaction was carried out at 25 °C in pH 5.11 (a) or 7.93 (b) 9 : 1 (v/v) buffer-dioxane. Aliquots of reaction mixture withdrawn at indicated times were eluted on a C-18 analytical column using a mobile phase of 34 : 1 : 65 MeOH-dioxane-water at a flow rate of 0.8 mL min $^{-1}$. Detection was made spectrophotometrically at 210 nm. Ep: epoxide, HP: hydrolysis product, St: standard, *cis*-1,2-dihydroxyindane.

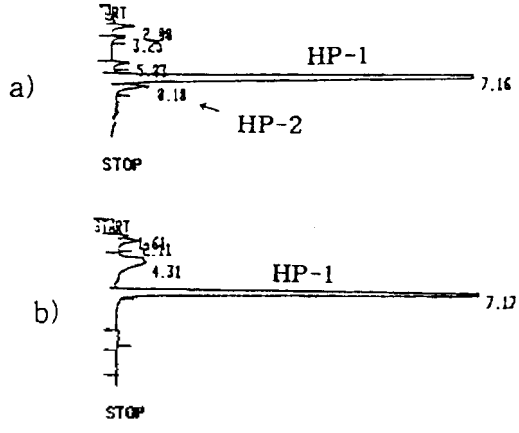
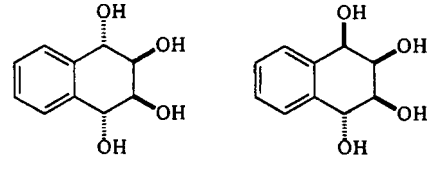


Figure 2. The HPLC product peak at the mobile phase of 5% MeOH from the hydronium-ion-catalyzed (a) and pH-independent (b) hydrolysis of epoxide **1**. See the legend of Figure 1 for the reaction conditions and HPLC procedures.

tion after complete epoxide disappearance was chromatographed using the mobile phase of 5% MeOH instead of 35% MeOH, the product peak was separated into two peaks (Figure 2(a)). The major product isolated by preparative HPLC has ^{1}H NMR spectrum with two symmetrical doublets with equal coupling constant (J=5.3 Hz) in addition to a symmetrical multiplet in the aromatic region. The symmetry in the spectrum indicates that the product itself possesses a C_2 axis or a plane of symmetry. The trans product (**5**) which has a plane of symmetry (an AA'BB' spin system) is consistent with the spectrum. The minor product was obtained in a quantity sufficient only for obtaining UV-Vis spectrum. The spectrum (λ_{max}=211 nm) resembled closely that of the major product (λ_{max}=210 nm). Thus, the minor product must be the *cis* product (**6**). No minor product was observed in the k_0 reaction (Figure 2(b)).

Hydrolysis of epoxide **2** yielded a substantial amount of a



5 **6**

minor product at both pH's (Figure 3). ^{1}H NMR spectrum of the major product isolated by HPLC consists of two symmetrical doublets of doublets at δ 3.47, 4.50 and in the aromatic region. The splitting pattern is consistent with the trans product (**7**) having a C_2 axis. The ^{1}H NMR spectrum of the minor product has two doublets at δ 4.71 (J=4.9 Hz) and δ 4.38 (J=8.2 Hz). From the splitting pattern and the

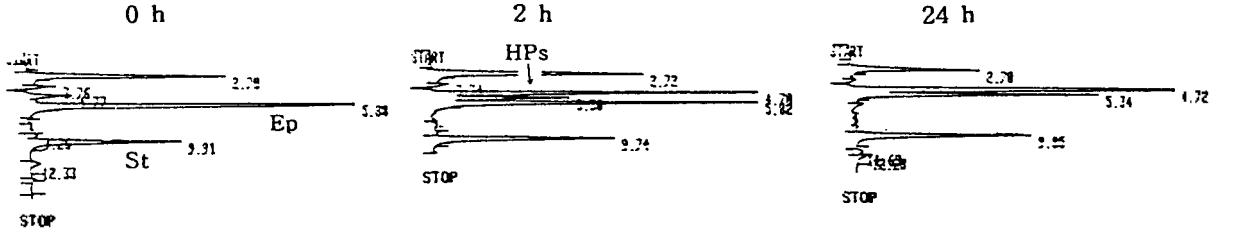
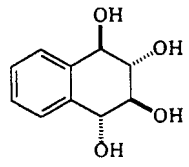
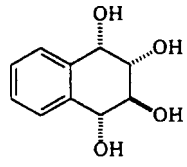


Figure 3. Time course by HPLC monitoring of the hydronium-ion-catalyzed hydrolysis of epoxide **2**. Reaction was carried out at 25 °C in pH 4.23 9 : 1 (v/v) buffer-dioxane. Aliquots of reaction mixture withdrawn at indicated times were eluted on a C-18 analytical column using a mobile phase of 34 : 1 : 65 MeOH-dioxane-water at a flow rate of 0.8 mL min $^{-1}$. Detection was made spectrophotometrically at 210 nm. Ep: epoxide, HPs: hydrolysis products, St: standard, *cis*-1,2-dihydroxyindane.

chemical shift values of C-2 and C-3 protons (δ 3.5), the two doublets must be benzylic protons. The spectrum is consistent with the cis product (**8**) having no symmetry element.

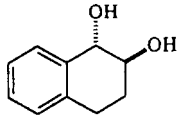


7

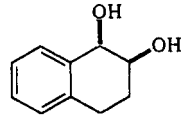


8

A small amount of a minor product is produced in the hydrolysis of epoxide **3** at both pH 6.58 and 7.93 (Figure 4). The major product isolated by preparative HPLC exhibited ^{1}H NMR spectrum with a doublet at δ 4.38 (J=7.2 Hz), whereas in the minor product, it is 2.5 Hz. While the vicinal coupling constant of the trans diol group in cyclohexenyl ring can be 2-10 Hz, that of the cis group can not be greater than 5 Hz.[15] Therefore, the major product is the trans product (**9**) and the minor one is the *cis* product (**10**).



9



10

Epoxide **4** was found to be quantitatively converted to 1-naphthol at both acidic and neutral pH's. Product identification was accomplished by coeluting the reaction mixture with 1-naphthol under various chromatographic conditions. Apparently, the tendency of this arene oxide to rearomatize by NIH shift[16] is too high for the carbocation trapping by water to compete.

The product distribution determined by HPLC for the hydrolysis of the epoxides are shown in Table 1.

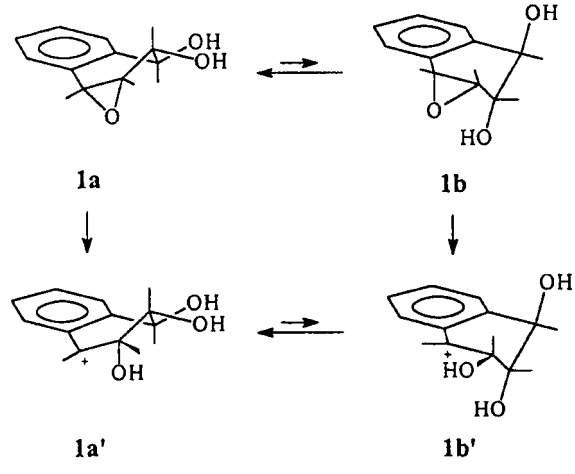
Because of a lack of data, the Jerina's proposal[10,11] of conformational effects in the hydrolysis of PAH diol epoxides could not be previously applied to the naphthalene diol epoxides. Now that the data have been obtained in this study, the proposal may be applied. For the benzo-ring diol epoxides in which hydroxyl groups are trans to each other, two rapidly interconvertable conformations are possible.[10] The two such conformations, the pseudodiequatorial (**1a**) and pseudodiaxial (**1b**) conformations of epoxide **1** are shown below. The carbocation formed by epoxide ring opening, likewise, would be in conformational equilibrium. The two conformations (**1a'** and **1b'**) of the carbocation formed by k_H reaction of epoxide **1** are shown below.

Table 1. Product distribution[a] in the hydrolysis[b] of epoxides **1-4**

Epoxide	pH	Trans	Cis	Epoxide	pH	Trans	Cis
1	5.11	98	2	**3**	6.58	95	5
	7.93	100	0		7.93	97	3
2	4.23	74	26	**4**	6.20	100	0
	7.93	87	13		7.93	100	0

[a] The yields of the products were determined by HPLC analysis of reaction mixtures. [b] Reaction was carried out at 25 °C in 9 : 1 (v/v) buffer-dioxane.

Since the predominant ground state conformation of epox-



1a **1b**

1a' **1b'**

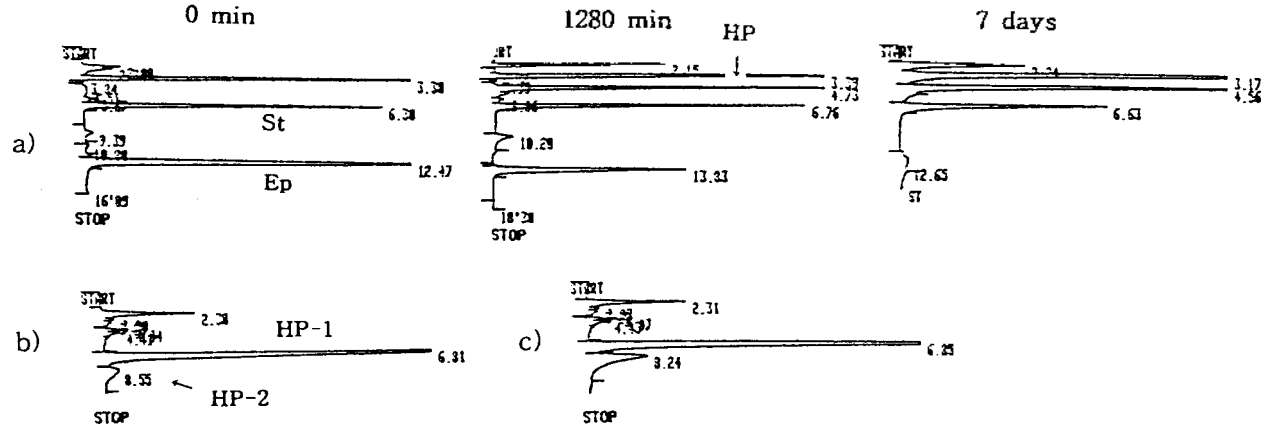
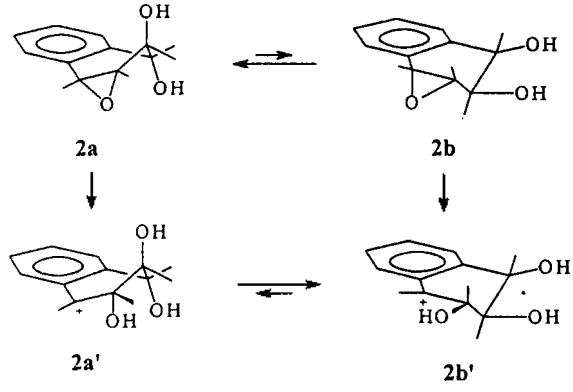


Figure 4. a) Time course by HPLC monitoring of the pH-independent hydrolysis of epoxide **3**. Reaction was carried out at 25 °C in pH 7.93 9 : 1 (v/v) buffer-dioxane. Aliquots of reaction mixture withdrawn at indicated times were eluted on a C-18 analytical column using a mobile phase of 44 : 1 : 55 acetonitrile-dioxane-0.01% Na_2HPO_4 at a flow rate of 0.8 mL min^{-1}. Detection was made spectrophotometrically at 210 nm. b) The product peaks at the mobile phase of 30% acetonitrile. c) The product peaks after addition of *cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene. Ep: epoxide, HP: hydrolysis product, St: 2-indanol.

ide **1** is **1a** ($J_{1,2}$=8.9 Hz) and **1a'** is calculated to be more stable than **1b'**,[10] epoxide ring opening should occur from **1a'**, and the diaxial approach of water to **1a'** from the top would give the trans product.

Epoxide **2** prefers conformation **2a** to **2b** ($J_{1,2}$=2.9 Hz). Of the two conformations of the carbocation from epoxide **2**, **2b'** is estimated to be more stable than **2a'**.[10]



The diaxial approach of water to this conformer would give the *cis* product. However, although the yield of the *cis* product from epoxide **2** is much higher than that from epoxide **1**, the trans product is still the major product. Hence, it is believed that the reaction begins largely from **2a'** and the carbocation formed is not so stable as to undergo facile conformational conversion.

It is difficult to predict the favorable conformation of epoxide **3** from its nmr data. However, exclusive trans hydration indicates that carbocation trapping by water occurs largely from the conformation in which hydroxyl group is axial.

It is observed that less *cis* product is formed in k_0 reactions of epoxide **1-3**. This could be attributed to the involvement of less stable zwitterionic intermediates and thus lesser probability of conformational conversion in k_0 process.

Acknowledgment. This research was made possible by the generous support of the National Institute of General Medical Sciences of U. S. A..

References

1. Phillips, D. H. *Nature* **1983**, *303*, 468.
2. Dipple, A. In *Polycyclic Hydrocarbons and Carcinogenesis*; Harvey, R. G. Ed.; ACS Sym. Ser. 283; American Chemical Society: Washington, D. C., 1985; p 1.
3. Harvey, R. G. *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*; Cambridge University Press: Cambridge, 1991; p 50.
4. Geacintov, N. E. In *Polycyclic Hydrocarbons and Carcinogenesis*; Harvey, R. G. Ed.; ACS Sym. Ser. 283; American Chemical Society: Washington, D. C., 1985; p 107.
5. Islam, N. B.; Gupta, S. C.; Yagi, H.; Jerina, D. M.; Whalen, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 6363.
6. Lee, Y. T.; Fisher, J. F. *J. Org. Chem.* **1993**, *58*, 3712.
7. Becker, A. R.; Janusz, J. M.; Rogers, D. Z.; Bruice, T. C. *J. Am. Chem. Soc.* **1978**, *100*, 3244.
8. Becker, A. R.; Janusz, J. M.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 5679.
9. Lee, Y. T. *Bull. Korean Chem. Soc.* **1993**, *14*, 412.
10. Sayer, J. M.; Yagi, H; Silverton, J. V.; Friedman, S. L.; Whalen, D. L.; Jerina, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 1972.
11. 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.
12. Lehr, R. E.; Kumar, S.; Levin, W.; Wood, A. W.; Chang, R. L.; Conney, A. H.; Yagi, H.; Sayer, J. M.; Jerina, D. M. In *Polycyclic Hydrocarbons and Carcinogenesis*; Harvey, R. G. Ed., ACS Sym. Ser. 283; American Chemical Society: Washington, D. C., 1985; p 63.
13. **1**: Yagi, H.; Thakker, D. R.; Hernandez, O.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 1604. **2**: reference # 5. **3**: Imuta, M.; Ziffer, H. *J. Org. Chem.* **1979**, *44*, 1351. **4**: Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3185.
14. Long, F. A.; Pritchard, J. G. *J. Am. Chem. Soc.* **1955**, *78*, 2663.
15. Abraham, R. J.; Gottschalck, H.; Paulsen, H.; Thomas, W. A. *J. Chem. Soc.* **1965**, 6268.
16. Jerina, D. M.; Daly, J. W. In *Oxidases and Related Redox Systems*; King, T. E.; Mason, H. S.; Morrison, M. Eds., Vol. 1; University Park Press: Baltimore, Maryland, 1973; p 143.