412 *Bull. Korean Chem. Soc., Vol. 14, No. 3, 1993*

Notes

Notes

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

Yong Tae Lee

*Department of Biochemistry, Yeungnam University,
Kyongsan 712-749*

The following epoxides, 1β,2α-dihydroxy-3α,4α-epoxy- (1) and 1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydronaphthalene (2) (*anti*- and *syn*-tetrahydrodiol epoxides), 1,2-epoxy-1,2,3,4-tetrahydronaphtalene (3), and 1,2-epoxy-1,2-dihydronaphthalene (4) are model compounds of the metabolites of carcinogenic polycyclic aromatic hydrocarbons (PAH'S).[1] In

1
(Epoxide I)

2
(Epoxide II)

3
(Epoxide III)

4
(Epoxide IV)
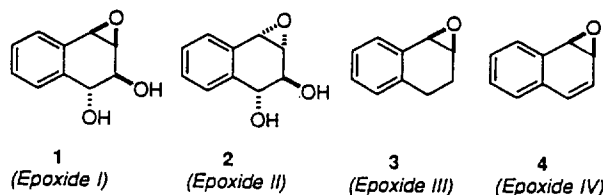
a study[2] undertaken by the author on the adduct formation between the above epoxides and reduced flavins such as $FMNH_2$, it was necessary to carry out the kinetic studies on the hydrolysis of these epoxides because hydrolysis competes with the adduct formation. Bruice *et al.*[3] reported previously on the kinetic study of the hydrolysis of the same epoxides under different conditions. In addition to the special importance of these compounds in the study of carcinogenesis of PAH's, the fact that one of the rate constants in their report is in sharp contradiction to the result of this study prompted the author to present his results.

A plot of the logarithm of the observed pseudo-first-order rate constant *vs.* pH always follows the rate expression[4]

$$k_{obsd} = k_H[H_3O^+] + k_0 + k_{OH}[OH^-] \qquad (1)$$

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. The k_{OH} process contributes only at very high pH. Accordingly, each of the three rate constants can be determined by performing kinetic measurements at the pH's where one among the three processes occur exclusively.

The k_H and k_0 values for the hydrolysis of epoxide I, II and III at 25°C in 9 : 1 (v/v) buffer-dioxane, determined by HPLC analysis of the reaction mixtures, are shown in Table 1. The buffers and pH values (buffer-dioxane) employed were; for k_H reactions, acetate (5.11), formate (4.23), and cacodylate (6.58) for epoxide I, II and III, respectively, and, for k_0 reactions, Tris (7.93) for all epoxides. The k_H values were also determined by spectrophotometry. Contribution of general catalysis by buffers was not examined because Bruice *et al.* found no buffer catalysis under similar condi-

Table 1. Rate Constants for the Hydronium-Ion-Catalyzed (k_H) and pH-Independent (k_0) Hydrolysis[a] of Epoxides I, II, and III

| Epoxide | k_H, M^{-1}s^{-1} | | | k_0, s^{-1} | |
	HPLC	Spectrophotometry	Literature[b]	HPLC	Literature[b]
I	1.1×10^1	1.1×10^1	4.0×10^1	2.2×10^{-6}	1.1×10^{-5}
II	1.7	2.2	6.0	1.8×10^{-6}	8.5×10^{-5}
III	3.1×10^2	3.5×10^2	1.2×10^3	1.0×10^{-5}	7.8×10^{-5}

[a] Reactions were run at 25°C in 9/1 (v/v) buffer-dioxane. [b] Reference 3. Determined at 30°C in 99/1 buffer-ethanol and ionic strength 1.0 M (KCl).
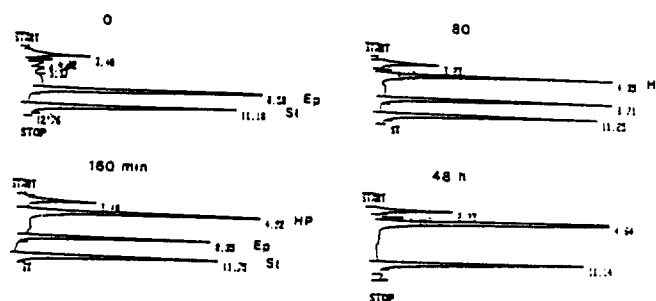

Figure 1. Time course by HPLC monitoring of the hydronium-ion-catalyzed hydrolysis of epoxide I. Reaction was carried out at 25°C in pH 5.11 9 : 1 (v/v) acetate-dioxane. Aliquots of reaction mixture withdrawn at 0, 80, 160 min and 48 h were eluted on a C-18 analytical column using a mobile phase of 34 : 1 : 65 methanol-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.

tions. The rate constants determined by Bruice *et al.* were included in the table for comparison. The reactions of epoxide IV at both pH 6.2 and 7.9 proceeded too fast to be monitored by HPLC method. The HPLC chromatograms of reaction aliquots during the hydrolysis of epoxide I at pH 5.11 are shown in Figure 1 and the HPLC procedures employed for epoxide I and II are described in the legend of the figure. The mobile phase used for the analysis of reactions of epoxide III was 44 : 1 : 55 CH_3CN-dioxane-0.01% Na_2HPO_4. The chromatographic changes observed in other reactions were similar. Rate constants were determined from the relative peak areas of the epoxides and hydrolysis products compared to the standards. The plot of $\ln C^0/C$ *vs.* time showed a reasonably good linear relationship (Figure 2). In the spectrophotometry, the Guggenheim plot[5] of the absorbance change observed in the reactions were used to obtain the k_H values. They also showed a good linear relationship.

The rate constants determined in this study by HPLC analysis, except k_0 for epoxide II, are in reasonable agreement
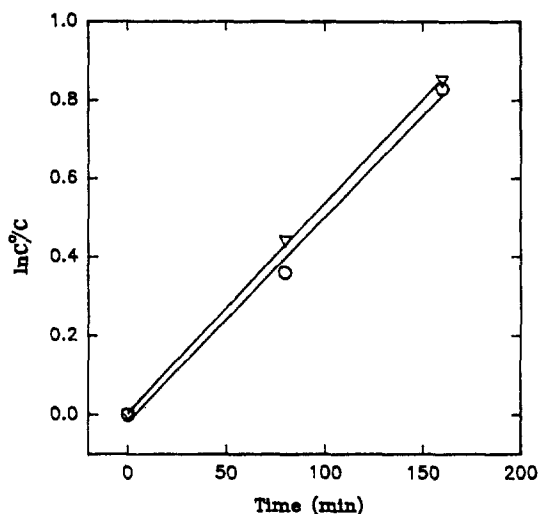
Figure 2. Plots of ln C^0/C vs. time for the hydronium-ion-catalyzed hydrolysis of epoxide I in pH 5.11 9 : 1 (v/v) acetate-dioxane at 25°C monitored by HPLC assay of both reactant (○) and product (△).

with those determined spectrophotometrically by Bruice *et al.*, The rates of the reactions in this study which are 3-8 times slower than those of Bruice *et al.* can be attributed to lower temperature and lower ionic strength. However, it is difficult to explain the k_0 value for epoxide II which is 47 times slower than that of Bruice *et al.*. HPLC method is increasingly used in kinetic studies. In several cases of kinetic studies on the hydrolysis of epoxides,[6] spectrophotometry was used for relatively fast hydronium-ion-catalyzed reactions and HPLC method was used for much slower spontaneous reactions. HPLC method would be a superior method for the investigation of the reactions that are very slow or show multiphasic kinetics resulting from the formation of secondary products. The k_0 reactions of epoxides I and II fall under this category. They are extremely slow (half-lives of epoxides I and II at neutral pH are 88 and 110 h respectively) and it is highly probable that epoxide II gives as a byproduct a keto diol rearrangement product[7] which must be quite unstable.[8] In the absence of specific steric constraints, only *syn*-diol epoxides such as epoxide II give substantial quantity of the keto diol products.[8,9]

As observed in other PAH system,[8,9] epoxide III, the tetrahydroepoxide, is considerably more reactive than the diol epoxides in both k_H and k_0 reactions. This could be attributed to rate-retarding inductive effect of hydroxyl groups in intermediate formation. It is not known whether ring opening, collapse of an intermediate or an unsymmetrical concerted process best describes the rate-limiting transition state for theses reactions.[10] However, evidence exists for the dependence of the solvolytic reactivity of PAH epoxides on the ease of formation of a cation at the benzylic position of the epoxide.[11]

In contrast to the similar reactivities in k_0 reactions, epoxide I is 6.5 times faster than epoxide II in k_H reaction. Qualitatively, this pattern of reactivity in k_H reactions is observed also in all of the PAH systems investigated to date.[6(a),8,9] There has been no satisfactory explanations for this trend of reactivity.[12] According to the pmr data ($J_{1,2}$ for

epoxide I = 9.0 Hz, $J_{1,2}$ for epoxide II = 3.0 Hz[13]), epoxide I and II are largely in the following conformations.

Epoxide I   **Epoxide II**
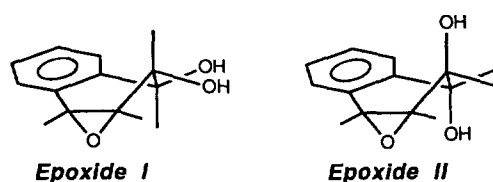
As observed in glycopyranose ring systems,[14] epoxide I in the ground state may have a destabilizing interaction between the epoxide oxygen and the adjacent hydroxyl group resulting from the unfavorable (parallel) orientation of electric fields generated by the two groups, which would decrease the activation energy for epoxide opening. Such an interaction is absent in epoxide II. A factor that may also affect the activation energy is 1,3-diaxial interaction between the C-1 and C-3 hydroxyl groups in the carbocation intermediate, which is existent only in the carbocation from epoxide II. This would increase the activation energy for epoxide opening of epoxide II.

References

1. (a) D. H. Philipps, *Nature*, **303**, 468 (1983); (b) R. G. Harvey, Ed. "Polycyclic Hydrocarbons and carcinogenesis" ACS Sym Series 283; American Chemical society: Washington, D. C., 1985; (c) R. G. Harvey, Ed. "Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity" Cambridge University Press: Cambridge, p. 50-95 (1991).
2. The manuscript was submitted to *J. Org. Chem.*.
3. (a) A. R. Becker, J. M. Janusz, D. Z. Rogers, and T. C. Bruice, *J. Am. Chem. Soc.*, **100**, 3244 (1978); (b) A. R. Becker, J. M. Janusz, and T. C. Bruice, *J. Am. Chem. Soc.*, **101**, 5679 (1979).
4. F. A. Long and J. G. Pritchard, *J. Am. Chem. Soc.*, **78**, 2663 (1955).
5. J. W. Moore and R. G. Pearson "Kinetics and Mechanism" 3rd ed. Wiley: New York, p. 71 (1981).
6. (a) J. M. Sayer, H. Yagi, M. Croisy-Delcey, and D. M. Jerina, *J. Am. Chem. Soc.*, **103**, 4970 (1981); (b) J. M. Sayer, R. E. Lehr, S. Kumar, H. Yagi, H. J. C. Yeh, G. M. Holder, C. C. Duke, J. V. Silverton, C. Gibson, and D. M. Jerina, *J. Am. Chem. Soc.*, **112**, 1177 (1990).
7. G. D. Guroff and D. M. Jerina, *Science*, **157**, 1524 (1967).
8. J. M. Sayer, D. L. Whalen, S. L. Friedman, A. Paik, H. Yagi, K. P. Vyas, and D. M. Jerina, *J. Am. Chem. Soc.*, **106**, 226 (1984).
9. D. L. Whalen, A. M. Ross, H. Yagi, J. M. Karle, and D. M. Jerina, *J. Am. Chem. Soc.*, **100**, 5218 (1978).
10. J. M. Sayer, H. Yagi, J. V. Silveron, S. L. Friedman, D. L. Whalen, and D. M. Jerina, *J. Am. Chem. Soc.*, **104**, 1972 (1982).
11. J. M. Sayer, R. E. Lehr, D. L. Whalen, H. Yagi, and D. M. Jerina, *Terahedron Lett.*, 4431 (1982).
12. Jerina *et al.* has proposed a hypothesis to rationalize the reactivity differences between the *syn*- and *anti* diol epoxides; see reference 10. However, the hypothesis fails when applied to k_H reactions.
13. H. Yagi, O. Hernandez, and D. M. Jerina, *J. Am. Chem. Soc.*, **97**, 6881 (1975).
14. R. U. Remieux and S. Koto, *Tetrahedron*, **30**, 1933 (1974).