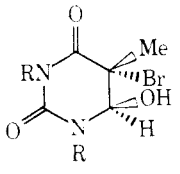
Regiospecific Oxygen Rearrangement in Reactions of Thymine Bromohydrins with Nucleophiles Using Bases: Formation of Thymine Epoxide Intermediates

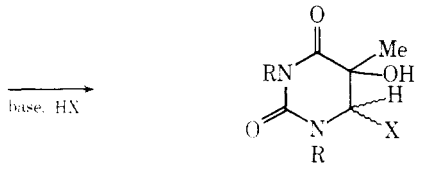
Summary: Studies of the reactions of *trans*-5-bromo-6-hydroxy-5,6-dihydrothymines with nucleophiles in the presence of bases provide more definitive evidence for the formation of pyrimidine epoxide intermediates, which react readily with nucleophiles such as water, methanol, and *N*-acetyltyrosine ethyl ester to yield thymine glycol derivatives via regiospecific oxygen rearrangement, and suggest that such epoxides might cause an alternative type of nucleic acid–protein cross-linkage in biological systems.

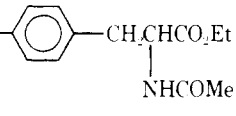
Sir: In recent years major attention has focused on the significant role of organic epoxides in biological systems as well as their chemical reactivity.[1] Previously we have presented results which suggest the formation of pyrimidine epoxides as initial oxidation products when pyrimidines are photochemically oxygenated using α-diketones as sensitizers.[2] Herein we describe reactions of *trans*-5-bromo-6-hydroxy-5,6-dihydrothymines **1a,b**[3] with nucleophiles in the presence of bases and provide more definitive evidence concerning the existence and the role of such intermediates.

Reaction of **1a** (1 mmol) with Et_3N (1 mmol) in THF (20 mL) containing H_2O (1 mL) at room temperature for 24 h under nitrogen atmosphere gave the corresponding glycols **2a** (cis and trans) (ratio cis/trans = 4:1) in a quantitative yield.[4] Treatment of **1a** with pyridine under similar conditions resulted in recovery of the starting material. However, **2a** (cis) was obtained in a quantitative yield when the reaction was carried out in water. Treatment of **1b** with pyridine in water similarly gave the corresponding glycol **2b** (cis) in a quantitative yield.[5] When **1a,b** were treated with Et_3N in methanol, the respective methanol adducts (**2c,d**) were formed quantitatively. Two isomers, **2c** (cis and trans)[6] (ratio 1:1), were obtained from **1a** whereas only one isomer, **2d**,[7] was produced from **1b.** Mass and ^1H NMR spectra of these adducts indicate that the methoxy group is incorporated at C-6 rather than at C-5, thus suggesting that oxygen migration occurs during the reaction.



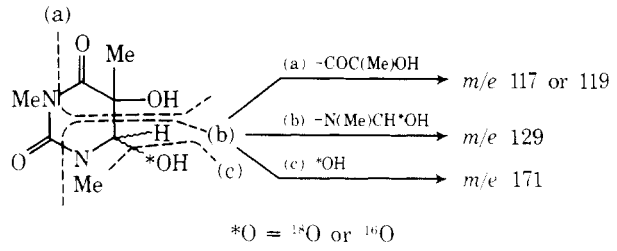
1a, R = Me
b, R = H



2a, R = Me; X = OH
a′, R = Me; X = ^{18}OH
b, R = H; X = OH
c, R = Me; X = OMe
d, R = H; X = OMe
e, R = Me; X = 

To obtain a more definitive evidence for oxygen migration, chemical trapping using $^{18}OH_2$ (60% purity) was carried out. The glycol **2a′** derived from the treatment of **1a** (0.25 mmol) with Et_3N (0.25 mmol) in THF (20 mL) containing $^{18}OH_2$ (0.2 mL) under similar conditions was analyzed by mass spectrometry.[8] Parent peaks at *m/e* 190 $(M + 2)^+$ and 188 (M^+) with an abundance ratio of $(M + 2)^+/M^+ = 1.8:1.0$ showed that only one ^{18}O was incorporated in each isomer. The other major fragment peaks were at *m/e* 171, 129, 119, and 117, and the relative abundance ratio of *m/e* 119 to *m/e* 117 was 1.9:1.0. No peak was observed at *m/e* 173 or *m/e* 131. On the other hand the mass spectrum of **2a** showed major peaks at *m/e* 188 (M^+), 171, 129, and 117. Interpretation of these data, summarized in Scheme I according to the known fragmentation pathways of 5,6-dihydropyrimidine glycols,[9] is consistent with the presence of incorporated ^{18}O in **2a′** at the C-6 position only. Any incorporation of ^{18}O at the C-5 position would give rise to peaks at *m/e* 173 and 131 in the spectrum of **2a′.**

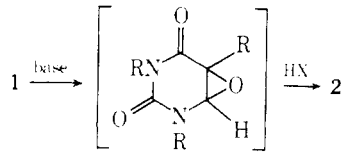
Scheme I



*O = ^{18}O or ^{16}O

This result constitutes proof of oxygen migration from C-6 to C-5 in the course of the reaction and strongly supports the initial formation of unstable thymine epoxide intermediates **3**, which subsequently react with nucleophiles to give the final products. The observed regiospecificity of the ring opening might be attributed to the contribution of the lone pair electrons on N-1 as proposed in a previous paper.[2]

High susceptibility of the intermediates **3** to nucleophiles such as -OH or -SH prompted us to examine the reaction with *N*-acetyltyrosine ethyl ester **4**. Similar treatment of **1a** with



3

Et_3N in THF in the presence of excess **4** led to formation of a single adduct **2e,**[10] which was separated by TLC on silica gel. No other isomer was detected.

With regard to nucleic acid–protein interaction, photochemical formation of nucleic acid–protein cross-linkages has been extensively investigated and several chemical mechanisms have been proposed.[11] Formation of **2e** strongly suggests that pyrimidine epoxides formed either by photooxidation or by chemical oxidants might cause an alternative type of nucleic acid–protein cross-linkages. The latter route may have added significance because a study using a dilute aqueous solution of radioactive $HO^{36}Cl$ has shown that the highest rate of inactivation was at pH 5.6, where 87% of ^{36}Cl bound to f2 bacterial virus was located in the RNA.[12]

The reaction mechanism including stereochemistry and other extensions of our system are being pursued.

References and Notes

(1) (a) G. J. Kasperek, T. C. Bruice, H. Yagi, N. Kaubisch, and D. M. Jerina. *J. Am. Chem. Soc.*, **94,** 7876 (1972); (b) J. W. Daly, D. M. Jerina, and B. Witkop, *Experientia,* **28,** 1129 (1972); (c) T. C. Bruice and P. Y. Bruice, *Acc. Chem. Res.*, **9,** 378 (1976), and references cited therein; (d) J. M. Hamman and H. H. Seliger, *Biochem. Biophys. Res. Commun.*, **70,** 675 (1976); (e) H. Kasai, K. Nakanishi, K. Frenkel, and D. Grunberger, *J. Am. Chem. Soc.*, **99,** 8501 (1977); (f) S. M. Kupchan, Y. Shizuri, T. Murae, J. G. Sweeny, H. R. Haynes, M.-S. Shen, J. C. Barrick, R. F. Bryan, D. van der Helm, and K. K. Wu, *ibid.*, **98,** 5719 (1976); (g) S. M. Kupchan, B. B. Jarris, R. G. Dailey, Jr., W. Bright, R. F. Bryan, and Y. Shizuri, *ibid.*, **98,** 7092 (1976); (h) J. T. Chan and H. S. Black, *Science,* **186,** 1216 (1974), and references cited therein.

0022-3263/79/1944-1191$01.00/0 © 1979 American Chemical Society

(2) H.-S. Ryang and S. Y. Wang, *J. Am. Chem. Soc.*, **100,** 1302 (1978).
(3) **1a,b** were synthesized according to the procedure of O. Baudisch and D. Davidson [*J. Biol. Chem.* **64,** 233 (1925)]. This method is known to give *trans*-bromohydrins: see S. Y. Wang, M. Apicella, and B. R. Stone, *J. Am. Chem. Soc.*, **78,** 4180 (1956); S. Y. Wang, *J. Org. Chem.*, **24,** 11 (1959); R. T. Teoule, B. Fouque, and J. Cadet, *Nucleic Acid Res.*, **2,** 487 (1975); D. Lipkin and J. A. Rabi, *J. Am. Chem. Soc.*, **93,** 3309 (1971).
(4) **2a** (cis and trans) was identified by comparison with authentic samples: see L. R. Subbaraman, J. Subbaraman, and E. J. Behrman, *J. Org. Chem.*, **38,** 1499 (1973).
(5) **2b** (cis) was identified by comparison with an authentic sample: see ref 4. Treatment of **1b** with Et_3N in CH_3CN or H_2O gave the corresponding salt (mp, 237–242 °C dec) instead of **2b.** The salt did not give **2b** even under reflux in H_2O.
(6) The products were analyzed as a mixture: molecular distillation 130 °C/1 mm (bath temperature); *m/e* 202 (M^+); NMR ($CDCl_3$, Me_4Si) of one isomer of **2c,** δ 1.41 (3 H, s, C_5-Me), 3.20 (6 H, s, NMe), 3.52 (3 H, s, OMe), and 4.26 (1 H, s, C_6-H); NMR of the other **2c,** δ 1.48 (3 H, s, C_5-Me), 3.20 (6 H, s, NMe), 3.48 (3 H, s, OMe), and 4.26 (1 H, s, C_6-H). Reflux of the mixture in MeOH did not change the ratio. However, heating of the mixture at 160 °C led to the decomposition of the latter isomer.
(7) **2d:** mp 195 °C (from CH_3CN); *m/e* 174 (M^+), 143 ($M - OCH_3$)$^+$, 115 ($M - NHCHOCH_3$)$^+$, 103 ($M - COCH_3OH_2$)$^+$, 101 ($M - COCH_3O$)$^+$, 72 ($COCCH_3OH$)$^+$; NMR (Me_2SO-d_6, Me_4Si) δ 1.40 (3 H, s, C_5-Me), 3.16 (6 H, s, NMe), 4.13 (1 H, d, J = 4.0 Hz, C_6-H), 5.32 (1 H, s, N_3H), 8.52 (1 H, d, J = 4.0 Hz, N_1H), 10.04 (1 H, s, —OH).
(8) Since cis and trans isomers of **2a** basically showed the same mass fragmentation pattern, mass spectrum of **2a'** was taken as a mixture of two isomers.
(9) C. Fenselau in "Photochemistry and Photobiology of Nucleic Acids", Vol. 1, S. Y. Wang, Ed., Academic Press, N.Y., 1976, Chapter 9, p 434.
(10) **2e** is a viscous liquid: molecular distillation 160 °C/1 mm (bath temperature); *m/e* 421; NMR ($CDCl_3$, Me_4Si) δ 1.24 (3 H, t, J = 8.0 Hz, —OCH_2CH_3) 1.51 (3 H, s, C_5-Me), 1.97 (3 H, s, COMe), 2.98 (3 H, s, NMe), 3.01 (2 H, d, J = 7.3 Hz, –CH_2–), 3.20 (3 H, s, NMe), 3.57 (1 H, s, OH), 4.11 (2 H, q, J = 8.0 Hz, –OCH_2CH_3), 4.77 (1 H, d + d, J = 8.7 and 7.3 Hz, –CH_2CHNH–), 4.97 (1 H, s, C_6-H), 5.94 (1 H, J = 8.7 Hz, NH), and 6.92 (4 H, m, arom).
(11) (a) K. C. Smith in "Photochemistry and Photobiology of Nucleic Acids. Biology", Vol. 2, S. Y. Wang, Ed., Academic Press, N.Y., 1976, Chapter 5, p 187; (b) M. P. Gordon, C. W. Huang, and J. Hurter, *ibid.*, Chapter 7, p. 270; (c) K. C. Smith, in "Aging, Carcinogenesis, and Radiation Biology", K. C. Smith, Ed., Plenum Press, N.Y., 1976, p 67; (d) P. R. Schimmel, G. P. Budzik, S. S. M. Lam, and H. J. P. Schoemaker, *ibid.*, p 123; (e) J. Sperling and A. Havron in "Excited States in Organic Chemistry and Biochemistry", B. Pullman and N. Goldblum, Eds., Dordrecht, Holland, Reidel Publishing, 1977, p 79, and references cited therein.
(12) W. H. Dennis, V. P. Olivieri, and C. W. Kruse, 175th National Meeting of the American Chemical Society, Anaheim, Calif., March, 1978.
(13) Department of Chemistry, University of California at Los Angeles, Los Angeles, California.
(14) This study was supported by NIH (R01-GM21146, R01-GM24238) and by DOE. This publication is identified as No. C00-3286-22.

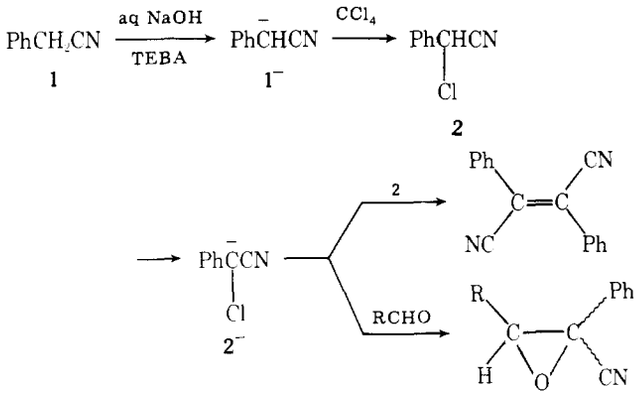
Hong-Son Ryang,[13] Shih Yi Wang*[14]
Program in Environmental Chemistry
Department of Environmental Health Sciences
School of Hygiene and Public Health
The Johns Hopkins University
Baltimore, Maryland 21205
Received December 12, 1978

Reactions of Carbon Tetrachloride with Carbon Acids in Catalytic Two-Phase System[1]

Summary: Phenylacetonitrile, its α-substituted derivatives, fluorene, and trichloroethylene react with carbon tetrachloride in the presence of 50% aqueous sodium hydroxide and triethylbenzylammonium chloride as catalyst to form chloro derivatives. The fate of these derivatives depends on the structure of the carbon acid. Carbanions of some of the chloro compounds formed in situ were trapped by suitable electrophiles to give derivatives of glycidic nitrile or dicyanocyclopropane, for example.

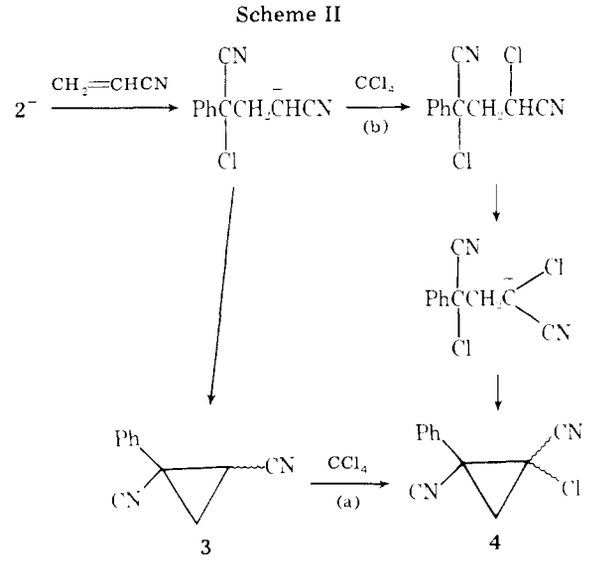
Sir: We have previously shown[2] that phenylacetonitrile (1) reacts with carbon tetrachloride in the presence of concentrated aqueous sodium hydroxide and triethylbenzylammonium chloride (TEBA) (socalled catalytic two-phase CTP system[3]) giving (*E*)-dicyanostilbene. The reaction proceeds via chlorination of 1 α-anion (1^-), and further transformations of phenylchloroacetonitrile (2) (Scheme I). Reactions between CCl_4 and some arylacetonitriles in solid potassium hydroxide/*tert*-butyl alcohol system were subsequently studied by Foucaud et al.,[4] who proposed an electron transfer as a step in these processes. Finally Meyers et al.,[5] on the basis of thorough studies of reactions of CCl_4 and other perhalomethanes with sulfones and ketones, have elaborated on the general mechanistic scheme of these processes which, they suggest, proceed via a radical/anion–radical pair (RARP) pathway.

Scheme I



Our detailed examination of the reaction between 1 and CCl_4 suggested the idea that the intermediate 2 α-anion (2^-) could be trapped by a suitable electrophile. This supposition was fully confirmed in the Darzens condensation with aldehyde. Thus stirring of 1, benzaldehyde, and CCl_4 with aqueous sodium hydroxide and TEBA resulted in an exothermic reaction leading to 1,2-diphenylglycidic nitrile (9:1 trans–cis mixture) in an isolated yield of 65%.[6] Similar reaction takes place with other aldehydes, for example isobutyraldehyde (Scheme I). One could expect that another active electrophile–acrylonitrile would also be able to trap the intermediate 2^-, with the formation of 1,2-dicyano-1-phenylcyclopropane (3). This process indeed takes place, resulting in the formation of two main products in combined yield 51%. However, the major component of the mixture was not 3, but 1,2-dicyano-1-chloro-2-phenylcyclopropane (4) (3 to 4 ratio was 1:4)

Scheme II



0022-3263/79/1944-1192$01.00/0 © 1979 American Chemical Society