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Mechanistic Studies on DNA Photolyase. 9. Is the Cleavage of the Cyclobutane Pyrimidine Photodimer Radical Anion a Pericyclic Reaction?

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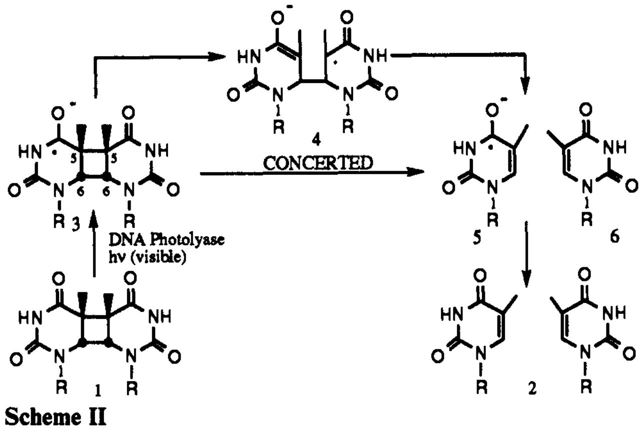
The enzymatic cleavage of the cyclobutane–pyrimidine photodimer **1** (Scheme I) plays a key role in protecting bacteria from the mutagenic and lethal effects of the UV component of sunlight.[1]

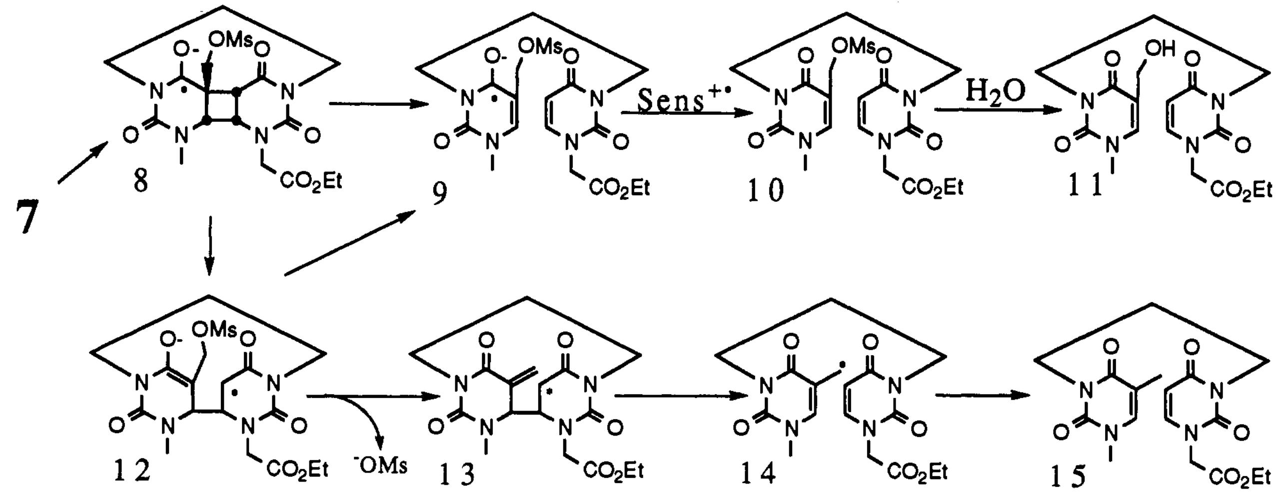
Scheme I



Isotope effect studies[2,3] and thermodynamic arguments[4] suggest that this reaction occurs by an initial electron transfer from the photoexcited enzyme-bound dihydroflavin to the photodimer. Two mechanisms for the fragmentation of the resulting photodimer radical anion **3** have been considered (Scheme I).[2,5] The first, based on the well-precedented cleavage of the methylcyclobutyl radical, involves the cleavage of the 5,5 bond to give the enolate radical **4**, followed by cleavage of the 6,6 bond. The second mechanism involves a concerted cleavage of the photodimer radical anion. While there are several other examples of formal retro [2 + 2] cycloadditions that are dramatically accelerated by 1-electron reduction, a pericyclic mechanism has not been experimentally established for any of these.[6] These precedents, however, combined with the isotope effect studies, have compelled us to seriously consider a pericyclic mechanism for the reaction catalyzed by DNA photolyase. In this communication, studies designed to trap the putative 1-bond-cleaved intermediate **4** in a model system, using a fast enolate trap, are described.

The departure of strategically placed leaving groups has been frequently used as a carbanion trapping strategy in mechanistic enzymology.[7] This suggests that photodimer **7** (Scheme III) should be a very sensitive probe for the 1-bond-cleaved intermediate **4**. For the stepwise mechanism in Scheme II, cleavage of the 5,5 bond of **8** would give enolate **12**. Cleavage of the 6,6 bond of **12** followed by back electron transfer to the sensitizer and hydrolysis of the highly reactive mesylate would give **11**. In competition with this path (**12** → **11**), loss of mesylate from **12** would give **13**. β-Scission of the 6,6 bond followed by hydrogen atom abstraction would give **15**.[8] If the fragmentation of **8** is

Scheme II



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(1) For leading references, see: (a) Sancar, A. In *Advances in Electron Transfer Chemistry*; Mariano, P. E., Ed.; JAI Press: London, 1992; Vol. 2, pp 215–272. (b) Sancar, A.; Sancar, G. B. In *Annual Reviews of Biochemistry*; Richardson, C. C., Ed.; Annual Reviews, Inc.: Palo Alto, CA, 1988; Vol. 57, pp 29–68.

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(6) (a) Horner, M.; Hunig, S. *Liebigs Ann. Chem.* **1982**, *8*, 1409. (b) Stevenson, G. R.; Sedgwick, J. B.; Miller, R. *J. Phys, Chem.* **1982**, *86*, 2441. (c) Gerson, F.; Weidmann, B. *Helv. Chim. Acta* **1966**, *49*, 1837. (d) Takamuku, S.; Kigawa, H.; Miki, S.; Sakurai, H. *Chem. Lett.* **1979**, 797. (e) Dekker, J.; Martins, F.; Kruger, J.; Gossen, A. J. *Tetrahedron Lett.* **1974**, 3721. (f) Russel, G. A.; Dodd, J. R.; Ku, T.; Tanger, C.; Chung, C. S. *J. Amer. Chem. Soc.* **1974**, *96*, 7255. (g) vonGizycki, U. *Liebigs Ann. Chem.* **1971**, *753*, 1. (h) Mullen, K.; Huber, W. *Helv. Chim. Acta* **1978**, *61*, 1310.

(7) For a review, see: Silverman, R. B. *Mechanism-Based Enzyme Inactivation*; CRC Press: Boca Raton, FL, 1988; Vols. 1 and 2.

(8) In support of this pathway (**13** → **15**), we have generated radical **13** independently by reaction of the corresponding 5-iodomethyl-substituted photodimer with tributyltin hydride and a catalytic amount of AIBN in refluxing acetonitrile. HPLC analysis of the reaction mixture indicated the formation of **15** as the only product.

(9) Fishbein, J. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1988**, *110*, 5087.

(10) A solution of **7** (1 mg/mL) and *N*-phenylpiperazine (5 equiv) in wet acetonitrile was degassed by purging with argon and irradiated at $\lambda > 300$ nm for 6 h. Under these conditions, **7** was not hydrolyzed, and no cleavage was observed in the absence of the sensitizer. The reaction mixture was then analyzed by reversed-phase HPLC (25% acetonitrile/water with UV detection at 254 nm).

(11) To insure that **8** ($pK_a \approx 7$: Steenken, S.; Telo J. P.; Novias, H. M.; Candeias, L. P. *J. Am. Chem. Soc.* **1992**, *114*, 4701) is deprotonated under the reaction conditions, we have used *N*-phenylpiperazine ($pK_a \approx 9$: Enea, O.; Houngbossa, K.; Berthon, G. *Electrochim. Acta* **1972**, *17*, 1585) as the sensitizer. While these pK_a values have been determined in water, the quenching of dimethoxytoluene fluorescence by the photodimer in water and in wet acetonitrile are essentially the same, indicating that the photodimer radial anion is not significantly destabilized in the latter solvent (Hartzfeld, D. G.; Rose, S. D. *J. Am. Chem. Soc.* **1993**, *115*, 850).

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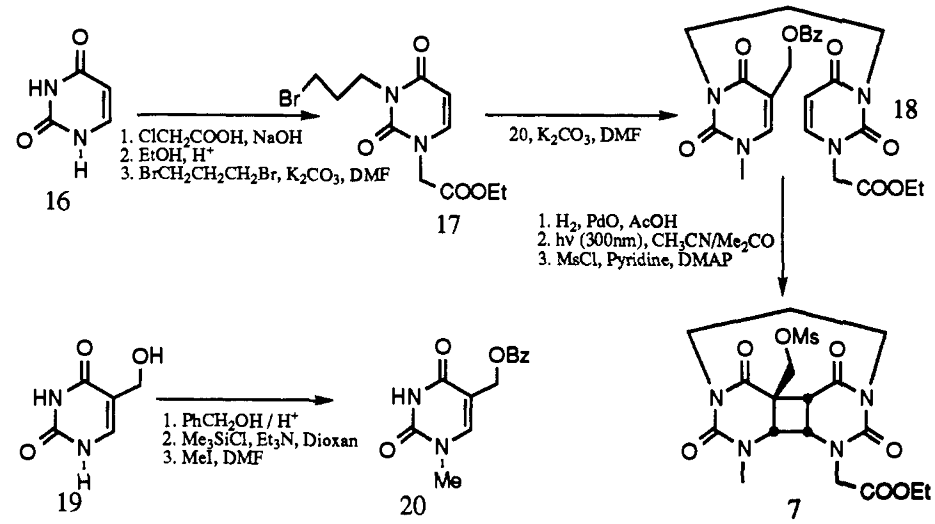
Scheme III



concerted (or if the rate of cleavage of the 6,6 bond of **12** is much faster than the rate of mesylate loss), **11** would be the only reaction product.

Thiophenolate expulsion from the anion of $(4\text{-NO}_2)C_6H_4\text{-}S(CH_2)_2CN$[9] occurs with a rate constant $\approx 10^{10}$ s^{-1}. While we have not measured the rate of loss of mesylate from **12**, this result indicates that it is likely to be very fast.

The synthesis of **7** is summarized in Scheme III. The photodimer radical anion **8** was generated by irradiation of a solution of **7** in wet acetonitrile in the presence of *N*-phenylpiperazine.[10,11] HPLC analysis of the reaction mixture demonstrated that cleavage occurred to give **11** (2–2.5%)[12] and that **15** was not formed (detection limit <0.01%).[13]

While proof of a concerted mechanism for the fragmentation of the photodimer radical anion will require an accurate rate constant for the departure of mesylate from **12**, the relatively large 6,6 isotope effect on the fragmentation of the photodimer radical anion[2,3] and the failure to trap the putative 1-bond-cleaved intermediate **4** using a fast enolate probe are consistent with the proposal that the photodimer radical anion fragmentation is a pericyclic reaction.[14]

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(12) Prolonged irradiation results in sensitizer decomposition and quenching of the cleavage reaction.

(13) Compound **15** was synthesized using chemistry analogous to that shown in Scheme III.

(14) We have attempted related experiments with the enzyme. The corresponding dinucleotide mesylate was not a substrate for the *Escherichia coli* DNA photolyase. The less sterically demanding 5-iodomethyl-substituted photodimers were mechanistically ambiguous due to the reductive lability of the C–I bond (Burdi, D. F. Ph.D. Thesis, Cornell University, 1992. Witmer, M. R. Ph.D. Thesis, Cornell University, 1990).