available reaction channels and the relative stability of the reaction products, thus possible causing the slight difference in the relative yield of the two isomeric furans.

Further work is in progress.

Experimental Section

^{19}F NMR spectra were recorded on a Bruker WH-400 NMR spectrometer. Mass spectra were recorded on an AEI MS 12 instrument, coupled to a 10% tricresylphosphate (6 mm × 4 m, 25 °C) gas-liquid chromatography column, and exact mass measurements were obtained on an AEI MS 50 spectrometer. Infrared spectra were recorded on a Nicolet 7199 fourier transform instrument, coupled to the same GLC column. Spectral data are summarized in Tables I, II, and III.

In the $\lambda > 335$ nm photolysis a medium-pressure mercury lamp (Hanovia #30620) with a window glass filter was used. In $\lambda = 254$ nm photolysis the source of radiation was a Hanovia low-pressure mercury resonance lamp with a 7910 Vycor filter.

A standard high-vacuum apparatus coupled to a gas-liquid chromatograph was employed to manipulate gases and isolate products for spectroscopic analysis.

Materials. Diazo ketones **1-3** were prepared according to the procedure reported for **1**.[26] Purification was carried out by preparative GC on a 10 ft × 1/4 in. i.d. SE-30 column at room temperature, and the spectral data are summarized in Table III.

(26) Dyatkin, B. L.; Mochalina, E. P. *Izv. Akad. Nauk. SSSR, Ser. Khim.* **1965**, *6*, 1035.

Exact $M^{\cdot+}$ mass spectral measurements for **2** and **3** were 255.98812 and 255.98818, respectively; the calculated value for $C_5F_8N_2O$ was 255.98828. The known s-*Z*/s-*E* conformational equilibrium of diazo ketones could be resolved for **1**, **2**, and **3** by low-temperature ^{19}F NMR as described in Table III and yielded an s-*Z*/s-*E* ratio of 1:4, 1:8, and 1:12, respectively.

Typical Photolysis Procedures. Precursor ketones with a sample pressure of 1.00-1.25 torr were placed in a 5 cm × 15 cm quartz cell with a 400-fold excess of hexafluoro-2-butyne. Photolysis times were varied from a few minutes to 16 h. Typical photolysis times were 2-3 at $\lambda = 254$ nm and 14 h at 335 nm. Determination of nitrogen and CO was used to monitor the extent of the photolysis and the absence of secondary photolysis of the ketene. Therefore, after each run the -195 °C noncondensible gases were measured in a gas buret followed by gas-liquid chromatographic analysis on a molecular sieve column (6 mm × 2 m, 25 °C). The reaction mixture was then distilled through two -98 °C traps. The condensate, which contained the products of interest, was analyzed by gas-liquid chromatography on the same tricresyl phosphate column and subjected to further spectroscopic analysis. The distillate, which contained the unreacted hexafluoro-2-butyne, was analyzed by gas-liquid chromatography using a medium activity silica gel column (6 mm × 3 m, 25 °C).

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Electron Delocalization in Pyrimidine Dimers and the Implications for Enzyme-Catalyzed Dimer Cycloreversion

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Pyrimidine dimers are cyclobutane derivatives produced by the photochemical cycloaddition reaction of two pyrimidines at their 5,6-double bonds. They are split by the photolyases, light-utilizing enzymes that may operate by a photoinduced, electron-transfer reaction that produces dimer radical anions. To assess the extent of charge delocalization in anionic species derived from dimers, we have measured base-catalyzed, cyclobutyl hydrogen exchange rates for a dimer in borate-buffered D_2O from pD 9.16 to 9.65. The kinetics were followed by NMR spectroscopy, and the rate constant for deuteroxide-catalyzed exchange, k_{OD}, was found to be 2.4×10^4 M^{-1} min^{-1}. The high rate of exchange supports the view that there is a carbanionic intermediate that is resonance-stabilized by electron delocalization from the cyclobutyl ring into the adjacent π system of the dimer. Simple Hückel molecular orbital theory has been applied to the π system of the dimer. The LCAO wave functions and approximate orbital energies have been derived. The Woodward-Hoffmann orbital symmetry conservation rules specify that the cycloreversion of the radical anion of cyclobutane is symmetry forbidden in the ground state. For the pyrimidine dimer radical anion, calculations showed that two cycloreversion mechanisms, a nonsynchronous concerted pathway and a totally nonconcerted pathway, proceed via transition states that are stabilized by a significant decrease in the energy of the orbital bearing the unpaired electron. A clearer view of why dimer radical anions are more prone to splitting than the neutral dimer has emerged from this study.

Pyrimidine dimers arise in DNA exposed to UV light as a consequence of a $(\pi_s^2 + \pi_s^2)$ photocycloaddition reaction between two stacked pyrimidines in the double helix.[1] The dimers, which are cyclobutane derivatives, exert deleterious effects in living systems,[2] many of which possess the ability to repair DNA photodamage of this type. Repair in a wide variety of living systems is accomplished, remarkably, by enzyme-catalyzed splitting of the cyclobutane ring of the dimer to regenerate pyrimidines.[3]

The light-requiring enzymes responsible for dimer splitting are the DNA photolyases.[4] These exceptional enzymes utilize light in the visible and near-UV range of the spectrum to accomplish pyrimidine dimer photocycloreversion.[5] The nature of the interaction of enzyme

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(2) Harm, W. *Biological Effects of Ultraviolet Radiation*; Cambridge University Press: London, 1980.

(3) Rupert, C. S. In *Molecular Mechanisms for Repair of DNA*, Part A; Hanawalt, P. C., Setlow, R. B., Eds.; Plenum Press: New York, 1975; pp 73-87.

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and DNA is becoming clear,[6] but the mechanism of dimer splitting remains unknown. It does not appear, however, to involve a simple, symmetry-allowed photocycloreversion to produce the original pyrimidines, because photoreactivating light is of insufficient energy to populate excited singlet or triplet states of typical dimers. Instead, photosensitized electron-transfer processes have been invoked to explain enzymatic[1b,7] and some nonenzymatic[8] splitting observations. Two distinct processes clearly involved in nonenzymatic systems are electron abstraction from the dimer and electron transfer to the dimer. The former generates a dimer radical cation, whereas the latter generates a dimer radical anion. These transient species are each prone to splitting. A subsequent electron-transfer step is required by both mechanisms to regenerate the original photosensitizer.

The dimer radical anion pathway is supported experimentally by the observations that solvated electrons,[8a] as well as electron donors like excited indoles,[9] are capable of splitting pyrimidine dimers. Recent studies have revealed that at least one photolyase may effect dimer splitting by the electron-donation process.[7] It is not known how the negative charge is distributed in the dimer radical anion, nor is it known how production of a dimer radical anion destabilizes the cyclobutyl ring toward splitting. Likewise unknown is whether the cycloreversion occurs by concerted and/or stepwise pathways and how the conservation of orbital symmetry influences the mechanism or rate of the enzyme-catalyzed cycloreversion.

We report here the base-catalyzed hydrogen exchange at the cyclobutyl ring of a pyrimidine dimer, an observation that implies the existence of an anionic intermediate. Simple frontier molecular orbital theory has been applied to the anion to rationalize the ease of the exchange reaction. This theory has also been applied to the dimer radical anion involved in dimer splitting to elucidate the manner in which addition of an electron to the dimer, whether by enzymatic or nonenzymatic processes, facilitates splitting. The mechanistic implications of the Woodward–Hoffmann rules for dimer radical anion cycloreversion have also been considered.

Experimental Section

General. Thymine, orotic acid (5-carboxyuracil), and isoorotic acid (6-carboxyuracil) were from Sigma Chemical Co. Synthesis of racemic *cis,syn*-1-(3-thymin-1-ylpropyl)-6-(*n*-butoxycarbonyl)uracil photodimer (**1**) was carried out as described.[8e] Column chromatography was carried out on E. Merck Kieselgel 60 (70–230 mesh). Me_2SO was allowed to stand overnight with activated alumina and KOH before use. For thin-layer chromatography, silica gel GF plates from Analtech, Inc. were used with the following solvent systems: A, H_2O–saturated ethyl acetate; B, 3% (by vol) methanol in ethyl acetate; and C, ethyl acetate–2-propanol–H_2O, 12:1:6 (by vol; upper phase). Melting points were determined with an Electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by MicAnal, Tucson, AZ.

Synthesis and Characterization of 2. The synthesis of racemic **2** was carried out by first preparing the precursor trimethylenebis(pyrimidine), 1-(3-thymin-1-ylpropyl)-5-(*n*-butoxycarbonyl)uracil, and then effecting photocycloaddition to **2**. Thus, the *n*-butyl ester of isoorotic acid and 1-(3-bromopropyl)thymine were coupled following the procedure for the synthesis of **1**.[8e] A solution of 1.00 g of *n*-butyl isoorotate and 0.233 g of 1-(3-bromopropyl)thymine in 20 mL of anhydrous Me_2SO was treated with 0.156 g of freshly dried and pulverized K_2CO_3 and allowed to stir at room temperature. After 16 h, thin-layer chromatography (solvent system A) showed residual *n*-butyl isoorotate (R_f 0.54) and the trimethylenebis(pyrimidine) (R_f 0.30) precursor of **2**. No residual 1-(3-bromopropyl)thymine (R_f 0.71) was detected. Solid potassium salts were removed by filtration and the filtrate was evaporated in vacuo. The resulting solid was resuspended in water-saturated ethyl acetate and applied to a dry silica gel column. Elution was carried out with water-saturated ethyl acetate. The trimethylenebis(pyrimidine) precursor of **2** obtained was recrystallized from acetone to yield 0.253 g (71%) of a white solid, mp 216–217 °C: R_f 0.17 (B), 0.27 (C); UV (95% EtOH) 277 nm (ϵ 2.28×10^4 M^{-1} cm^{-1}); ^1H NMR (Me_2SO-d_6) δ 0.90 (t, 3 H, $CH_3CH_2CH_2CH_2$), 1.37 (sextet, 2 H, $CH_3CH_2CH_2CH_2$), 1.61 (quintet, 2 H, $CH_3CH_2CH_2CH_2$), 1.74 (d, J = 0.8 Hz, 3 H, thyminyl-CH_3), 1.92 (m, 2 H, $NCH_2CH_2CH_2N'$), 3.66 and 3.81 (2 t, 4 H, $NCH_2CH_2CH_2N'$), 4.14 (t, 2 H, $OCH_2CH_2CH_2CH_3$), 7.53 (s, 1 H, Thy-C(6)*H*), 8.48 (s, 1 H, isoorotyl-C(6)*H*), 10.09 and 10.36 (2 br s, NH and N'H). Anal. Calcd for $C_{17}H_{22}N_4O_6$: C, 53.96; H, 5.86; N, 14.81. Found: C, 53.66; H, 5.79; N, 14.57.

Preparation of *cis,syn*-1-(3-thymin-1-ylpropyl)-5-(*n*-butoxycarbonyl)uracil photodimer (**2**) from its precursor was carried out as follows. A solution of 0.19 g of 1-(3-thymin-1-ylpropyl)-5-(*n*-butoxycarbonyl)uracil in 650 mL of water was prepared with gentle heat, 250 mL of acetone was added, and the solution was thoroughly purged with N_2. Irradiation was carried out in a quartz immersion well photoreactor (Ace glass) by use of a 450-W medium pressure Hg lamp (Hanovia) with a Kimax sleeve. During irradiation, the solution was well stirred and continuously purged with N_2. Photocycloaddition was judged to be complete after 3.0 h by the disappearance of the peak at 270 nm in the absorption spectrum of an aliquot of the reaction mixture that was dried and dissolved in water. The solution was evaporated to a volume of approximately 70 mL and the resulting white precipitate was collected and washed with a small amount of water to yield 0.155 g (83%) of **2**. Recrystallization from acetone followed by drying at reduced pressure over KOH gave analytically pure **2**: mp 310–314 °C; R_f 0.27 (B), 0.32 (C); UV (95% EtOH) λ_{max} <230 nm (ϵ 2.67×10^3 M^{-1} cm^{-1} at 240 nm); ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3 H, $CH_3CH_2CH_2CH_2$), 1.33 (m, 2 H, $CH_3CH_2CH_2CH_2$), 1.52 (s, 3 H, cyclobutyl-CH_3), 1.5–1.6 (m, 3 H, $CH_3CH_2CH_2CH_2$ and $NCH_2CHH'CH_2N'$), 1.84 (m, 1 H, $NCH_2CHH'CH_2N'$), 2.76 (m, 1 H, $NCHH'CH_2CHH'N'$), 2.96 (m, 1 H, $NCHH'CH_2CHH'N'$), 3.82 [d, J = 6.4 Hz, 1 H, cyclobutyl: "thyminyl-C(6)H"), 4.1–4.2 (m, 4 H, OCH_2 and $NCHH'CH_2CHH'N'$), 4.68 [d, J = 6.4 Hz, 1 H, cyclobutyl: "isoorotyl-C(6)H"], 10.4 (s, NH), 10.8 (s, N'H). Anal. Calcd for $C_{17}H_{22}N_4O_6$: C, 53.96; H, 5.86; N, 14.81. Found: C, 53.67; H, 5.98; N, 14.70.

NMR Spectroscopy. NMR spectra were recorded on a Brucker AM-400 pulsed spectrometer at 400 MHz or on a Brucker WH-90 spectrometer. Signal positions are reported in parts per million relative to an internal standard (tetramethylsilane for organic solvents and DSS for D_2O). To define the spectral changes accompanying the exchange reaction, a dimer spectrum was initially recorded in a mixture of acetone-d_6 and D_2O (9:1, v/v), then again after addition of NaOD to a final concentration of 5.6 mM,

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and a third time following neutralization with DCl (solution pD 5 to 6). Thin-layer chromatography (B) revealed that little or no ester hydrolysis occurred as a result of NaOD treatment.

Kinetics of Exchange. The kinetics of exchange were followed by ^1H NMR spectroscopy at a probe temperature of 25 ± 0.5 °C. A solution of dimer in D_2O (0.45 mL) was treated with 0.050 mL of 50 mM potassium borate in D_2O, prepared by repeated lyophilization of D_2O solutions of $B(OH)_3$. The NMR spectrum was recorded at various times following the addition of buffer. Data collection was facilitated by the use of an NMR kinetics automation program. Typically data acquisition required 4.3 min, and a delay of 1 to 3 min was allowed between successive spectra. Integrations of the signals due to the exchangeable cyclobutyl proton (I_x) and the nonexchangeable cyclobutyl proton (I_n), or the signal at δ 4.1–4.2 if the latter was obscured, were used in the kinetic analysis described below.

The acidity of each solution was measured after data collection by use of the glass electrode (Radiometer PHM 62 pH meter, electrode GK 2321C). Values for $[OD^-]$ were calculated according to the relationship

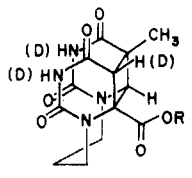
$$[OD^-] = (K_w^D)10^{pH+0.41}$$

where pH is the pH meter reading of the D_2O solution. The value of K_w^D was taken to be 1.5×10^{-15} M^2.[10]

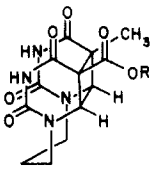
Molecular Orbital Calculations. A published computer program[11] for the calculation of simple Hückel MO eigenvalues and eigenvectors was adapted for use on a VAX/VMS system. Standard parameters were used to approximate the Coulomb and resonance integrals of the heteroatoms in the system (h_N = 1.5, k_N = 0.8; h_O = 1.0, k_O = 1.0).[12] Calculations for the transition states discussed below were carried out assuming *no* interaction between partially bonded, sp^2-hybridized carbon atoms. At this level of theory the nonsynchronous concerted and fully nonconcerted (i.e., stepwise) pathways are equivalent. No allowance was made for possible interactions of the π systems of the two "pyrimidines" of the dimer.

Results

Examination of the ^1H NMR spectrum of **1** revealed that the two cyclobutyl protons are weakly coupled (J = 1 Hz) and give rise to sharp doublets at 4.6 and 3.6 ppm [C(6)H of thymine and C(5)H of orotate, respectively; data not shown]. Upon addition of NaOD, the 3.6 ppm signal rapidly disappeared and the 4.6 ppm resonance became a singlet, indicating that exchange of the C(5) cyclobutyl proton had occurred. The spectrum recorded after neutralization with DCl was essentially identical with that measured in basic solution, except for small variations in chemical shift values due to differences in pD (data not shown). This provided assurance that the disappearance of the signal at 3.6 ppm was the result of exchange of a cyclobutyl hydrogen in **1**.



1



2

The same type of experiment was carried out with the isomeric dimer derived from thymine and isoorotate (5-carboxyuracil), **2**. In this compound, neither of the two cyclobutyl hydrogen atoms is α to a ring carbonyl group. The inductive effect of the β carbonyl group of the side-chain ester is common to both isomers. Upon treatment with NaOD, no change in the NMR spectrum was observed (data not shown). Thus, exchange of a cyclobutyl hydrogen atom under these mildly alkaline conditions required that the hydrogen atom be α to a carbonyl group and was not a consequence of the inductive effect of the more distant ester side chain. Stabilization by charge delocalization into an adjacent carbonyl group is invoked below to explain the ease of exchange.

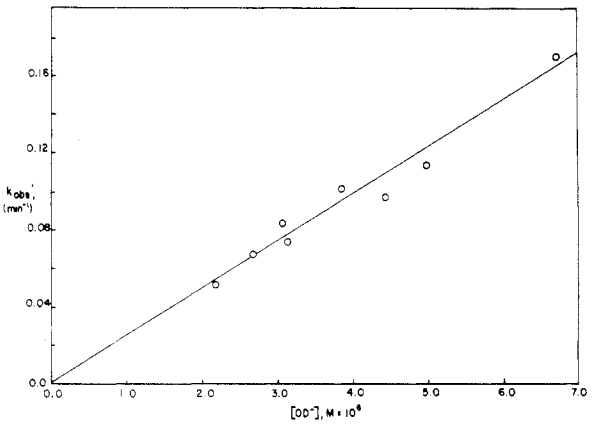


Figure 1. Kinetic data for cyclobutyl hydrogen exchange in **1**. Correction of k_{obs} for a small amount of buffer catalysis gave k_{obs}'.

Exchange was considered to proceed by deprotonation of the cyclobutyl group by OD^- to produce a carbanion in a rate-limiting, irreversible step, i.e., a step that could only lead to deuterium incorporation. This is consistent with exchange in other carbonyl-containing systems.[13] The mechanistic scheme involving catalysis by OD^- was

$$SH + OD^- \xrightarrow[\text{slow}]{k_{OD}} S^- \xrightarrow[\text{fast}]{D_2O} SD$$

The rate law for the exchange reaction was assumed to be

$$v = \{k_0 + k_B[B(OD)_4^-] + k_{OD}[OD^-]\}[SH]$$

The NMR integration data were plotted according to the relationship

$$\log(I_x/I_n) = -k_{obs}t/2.30$$

where k_{obs} is the following pseudo-first-order rate constant

$$k_{obs} = k_0 + k_B[B(OD)_4^-] + k_{OD}[OD^-]$$

To correct for the catalysis due to the buffer anion, $B(OD)_4^-$, the buffer constant, k_B, was estimated separately by measurement of the exchange rates at pD 9.3 at several concentrations of buffer (15 to 50 mM), with ionic strength held constant (I = 16 mM, with KCl). In this way k_B was found to be approximately 1.1 M^{-1} min^{-1}. This allowed k_{obs} to be corrected for buffer catalysis by use of k_B and the pK_a of $B(OD)_3$ in D_2O, which is 9.07.[14] Thus,

$$k_{obs}' = k_{obs} - k_B[B(OD)_4^-]$$

and

$$k_{obs}' = k_{OD}[OD^-] + k_0$$

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Table I. HMO Energy Levels for Pyrimidine Dimer and Hypothetical Splitting Transition States

species[a]	values of n for $E = \alpha + n\beta$							
D	2.45	1.86	1.34	1.16	−0.70	−1.11		
A*	2.49	1.95	1.37	1.17	0.40	−0.95	−1.42	
B*	2.54	2.04	1.50	1.18	−0.34	−0.75	−1.17	
C*	2.64	2.05	1.71	1.18	0.74	−0.65	−0.95	−1.72

[a] See Chart I for structures of these species.

A plot of k_{obs}' against [OD^-] is shown in Figure 1. The data confirmed first-order dependence of exchange on [OD^-]. The slope of the least-squares line through the data gave $k_{OD} = 2.4 \times 10^4$ M^{-1} min^{-1} ($r = 0.979$).

Correction for buffer catalysis deserves comment. Because k_B is small, there is considerable uncertainty in the determination of its value. Furthermore, the pK_a of $B(OD)_3$ in D_2O was determined at high ionic strength. These factors together make a correction of k_{obs} for buffer catalysis somewhat inaccurate. It was possible, however, to make a nearly independent estimate of k_{OD} by use of the buffer catalysis plot of k_{obs} against [$B(OD)_4^-$] (data not shown). The y intercept of this plot, 7.7×10^{-2} min^{-1}, provided the sum $k_0 + k_{OD}$[OD^-], which includes exchange due to the lyonium-catalyzed and "uncatalyzed" reactions (taken together as k_0) as well as the OD^--catalyzed reaction. Since the last of these is expected to be overwhelmingly larger than the former two, the intercept constitutes an estimate of k_{OD}[OD^-]. The buffer catalysis study was carried out at [OD^-] = 3.0×10^{-6} M (pD 9.3), so $k_{OD} \cong 2.6 \times 10^4$ M^{-1} min^{-1}, in good agreement with the value of 2.4×10^4 M^{-1} min^{-1} obtained from the plot of k_{obs}' against [OD^-].

Molecular Orbital Calculations. The results of the molecular orbital calculations are shown in Table I. The significance of these results is discussed below.

Discussion

Hydrogen Exchange. Base-catalyzed hydrogen exchange at the cyclobutyl ring of pyrimidine dimer 1 in mildly alkaline aqueous solution occurred readily, implying the existence of a carbanionic intermediate. The rate constant for OD^--catalyzed exchange was found to be 2.4×10^4 M^{-1} min^{-1}. This value is comparable to rate constants for deprotonation of β-dicarbonyl compounds by hydroxide ion in water. For example, the rate constants for OH^--catalyzed enolization of typical β-keto esters in aqueous solution are in the range of 6×10^3 to 2×10^5 M^{-1} min^{-1}.[15] Monoketones enolize some 4 orders of magnitude more slowly. For acetone the value of k_{OH} is 13 M^{-1} min^{-1}.[16] Thus, deprotonation of the dimer 1 occurs very readily.

It was found that 2, a structural isomer of 1, did not undergo an exchange reaction at the cyclobutyl ring. In 2 neither cyclobutyl hydrogen is α to a carbonyl group. The implication of this result is that hydrogen exchange at the cyclobutyl ring can only occur if the lone pair formed upon deprotonation can be delocalized into an adjacent carbonyl group, thereby stabilizing the carbanionic intermediate.

In principle, the carbonyl group α to the exchangeable hydrogen could facilitate carbanion formation by both inductive and resonance effects. Generally, however, the inductive effect of an α carbonyl group is insufficient to facilitate exchange in mildly alkaline solutions. Bridgehead hydrogens in bicyclic systems with an α carbonyl group, for example, are well-known to be unreactive toward exchange because overlap of the π system of the carbonyl group with the orbital bearing the lone pair formed on deprotonation is not possible.[17] The observed, base-catalyzed exchange of a cyclobutyl hydrogen in 1 is, therefore, indicative of delocalization of the lone pair of electrons of the carbanionic intermediate into the π system encompassing the α carbonyl group.

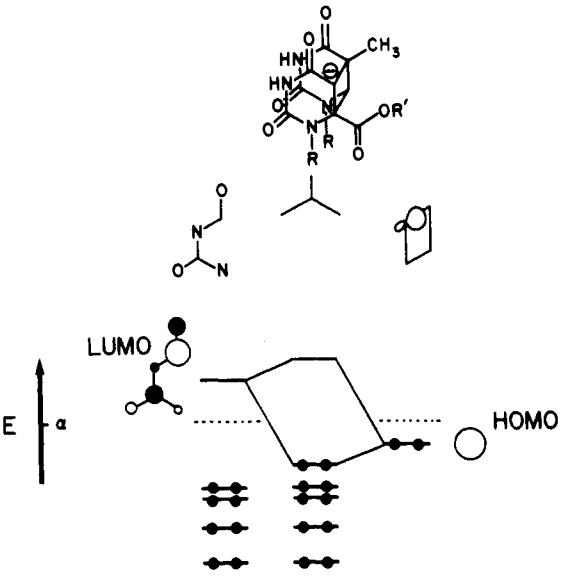


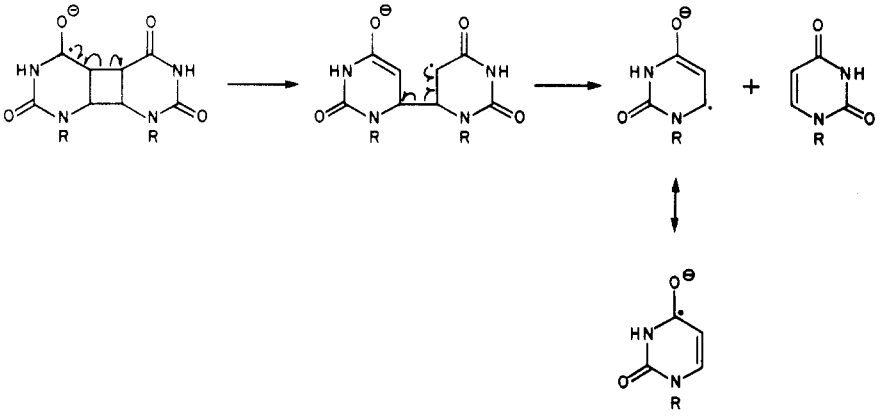
Figure 2. Frontier molecular orbital interaction diagram for the carbanion involved in hydrogen exchange. The LUMO has a major contribution from the carbonyl group adjacent to the cyclobutyl carbon bearing the lone pair of electrons in a hybrid atomic orbital, labeled HOMO. Stabilization is achieved through the large interaction afforded by the carbonyl carbon atom and the effects of the electronegative atoms participating in the LUMO. (Higher energy vacant orbitals are omitted for clarity.)

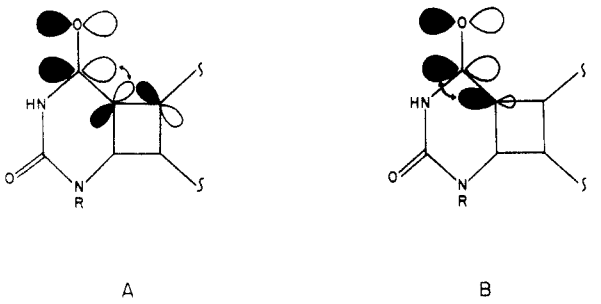
Charge delocalization in the hypothetical carbanionic intermediate in the exchange reaction can be rationalized by use of simple frontier molecular orbital theory. The carbonyl group is part of a π-orbital system that extends over several atoms of the dimer. By use of simple Hückel MO theory, an approximate energy level scheme for those π orbitals has been formulated. The phases and magnitudes of the participating p orbitals were also deduced in this manner. The resulting MO energy levels and the forms of the orbitals are shown in Figure 2. The adjacent cyclobutyl carbon atom bears a lone pair in a hybrid atomic orbital, labeled HOMO, whose energy is set somewhat lower than the $E = \alpha$ level because of the s character of the hybrid. Allowing the interaction of frontier orbitals produces a new pair of orbitals, whose energies are qualitatively shown. Net stabilization is achieved as a result of this interaction, since only the lower energy orbital is occupied. The magnitude of the stabilization cannot be quantitated by this simple theoretical framework. The stabilization is, however, facilitated by the large contribution of the carbonyl carbon atom's p orbital to the LUMO, which allows the lone pair to interact efficiently with the system of heteroatoms. This region of the anion undoubtedly flattens somewhat to increase the HOMO–LUMO overlap and consequently the extent of stabilization.

Pyrimidine Dimer Splitting. A perplexing question in DNA photorepair is the following: how does photoreduction of the dimer to the radical anion by an enzyme

(15) Guthrie, J. P. *Can. J. Chem.* **1979**, *57*, 1177–1185.
(16) Chiang, Y.; Kresge, J.; Tang, Y. S. *J. Am. Chem. Soc.* **1984**, *106*, 460–462.
(17) Gutsche, C. D. *The Chemistry of Carbonyl Compounds*; Prentice-Hall: Englewood Cliffs, NJ, 1967; pp 13–14.

Scheme I





A B

Figure 3. Arrangement of atomic orbitals in part of the dimer framework. The carbonyl carbon atom's p orbital is oriented so that it can interact with a hybrid atomic orbital used by a cyclobutyl carbon atom in a bond holding the components of the dimer together.

facilitate splitting? The reduction and splitting steps are represented below

$$\text{dimer} + e^- \rightarrow \text{dimer}^{\bullet -} \rightarrow \text{pyrimidine} + \text{pyrimidine}^{\bullet -}$$

Splitting of a pyrimidine dimer radical anion might, in general, occur by concerted and/or nonconcerted (i.e., stepwise) mechanisms. The former case can be delineated by extremes, namely, synchronous and nonsynchronous (but still concerted) pathways. The Woodward–Hoffmann rules governing conservation of orbital symmetry[18] are applicable to both such concerted transformations.

The splitting that pyrimidine dimer radical anions undergo may be rationalized by use of the molecular orbital scheme derived above for the exchange reaction. Transfer of an electron to the dimer results in addition of an electron to the orbital previously labeled LUMO. LUMO must be renamed SOMO to reflect the fact that it is now a singly occupied molecular orbital. There are, in principle, two ways that SOMO can weaken the bonds holding the constituent "pyrimidines" of the dimer together. One is for SOMO to draw electron density from cyclobutyl orbitals that are bonding in character. Alternatively, SOMO can induce the splitting by contributing electron density to a molecular orbital that has antibonding character across the cyclobutyl bonds linking the constituents of the dimer. In terms of valence-bond structures, these processes are equivalent to the first step of the splitting mechanism shown in Scheme I. The remaining steps shown in Scheme I are reasonable consequences of the initial bond breaking.

The interaction of a typical cyclobutyl ring orbital with SOMO is shown in Figure 3A. This interaction might in fact be quite extensive, because the physical orientation

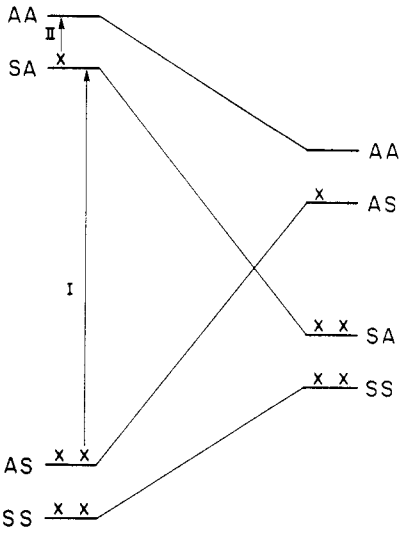


Figure 4. Orbital symmetry correlation diagram for cycloreversion of cyclobutane radical anion to ethylene and ethylene radical anion. The path beginning with excitation step I is symmetry allowed, but the one beginning with step II is symmetry forbidden. The ground state path (i.e., no excitation) is symmetry forbidden.

of the cyclobutyl bond orbital relative to SOMO appears to be much like the orientation of HOMO relative to LUMO, shown in Figure 3B. The interaction of the latter two orbitals is extensive enough to facilitate the hydrogen exchange reaction discussed above.

If indeed the unpaired electron is delocalized into an antibonding orbital of the cyclobutyl ring, a species is generated that has the character of a cyclobutane radical anion. The dimer anion cycloreversion reaction then resembles that of the cyclobutane radical anion. The orbital correlation diagram for the cycloreversion of cyclobutane radical anion is shown in Figure 4. Inspection of the diagram reveals that the thermal (i.e., ground state) pathway is symmetry forbidden. Of the two photochemical (i.e., excited state) pathways, one (labeled I) is symmetry allowed and one (labeled II) is symmetry forbidden. If this diagram is applicable to dimer radical anion cycloreversion, it leads to the conclusion that the "symmetry forbiddenness" of thermal dimer cycloreversion is *not* evaded by the formation of the dimer radical anion. The latter species is likewise symmetry forbidden to split by a thermal process, although the actual kinetics of splitting might be different for the neutral and anionic species (vide infra).

In spite of the fact that the dimer radical anion is formally forbidden by conservation of orbital symmetry to split by a concerted, ground-state process, the observed

(18) Woodward, R. B.; Hoffmann, R. *The Conservation of Orbital Symmetry*; Academic Press: New York, 1971.

(19) Harm, W. *Mutation Res.* **1970**, *10*, 277–290. Harm, H.; Rupert, C. S. *Mutation Res.* **1970**, *10*, 307–318.

Chart I

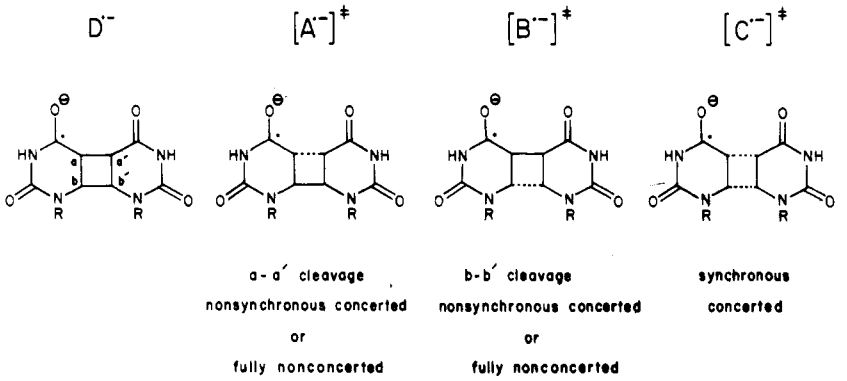
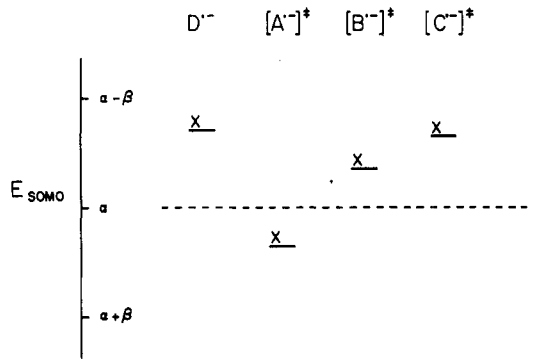


Chart II



efficiency of splitting in nonenzymatic cases is approximately 5%,[8a,e] which argues that splitting is inefficient but not "forbidden" in a kinetic sense. Indeed, the enzymatic reaction proceeds with a quantum yield of unity.[7,18] These results can be rationalized by considering the possible rate enhancement (relative to a fully concerted process) that could be achieved by a nonsynchronous concerted or even a totally nonconcerted cycloreversion mechanism.[20]

The reactant (dimer radical anion) as well as three hypothetical transition states for splitting are shown in Chart I. The three transition states arise by predominant cleavage of either the a–a′ bond, the b–b′ bond, or the fully concerted breaking of both the a–a′ and the b–b′ bonds of the cyclobutyl ring. In each case to be considered, the energy required for σ-bond cleavage is offset by the stability of the insipient π system. A kinetic acceleration of splitting will be caused by reduction of D to D•⁻ if the "extra" electron decreases the energy difference between D•⁻ and the anionic transition state *relative to D and the corresponding uncharged transition state.*

The Hückel molecular orbital energies of the SOMO of D•⁻ and each of the three hypothetical anionic transition states is shown in Chart II. Both D and the concerted mechanism's transition state C‡ are stabilized to about the same extent by addition of an electron to form D•⁻ and [C•⁻]‡ (0.70β and 0.65β, respectively). Thus, there is essentially no kinetic acceleration expected for the path via [C•⁻]‡ upon one-electron reduction of D. In striking contrast, the anionic transition state [A•⁻]‡ is 1.1β (0.70β + 0.40β) more stable relative to D•⁻ than A‡ is relative to D. This should dramatically accelerate splitting relative to the pathway involving neutral A‡ as well as the anionic concerted pathway. Finally, acceleration via [B•⁻]‡ is intermediate between these extremes, with the activation energy decreased by only 0.36β (0.70β − 0.34β) for the anions.

In summary, one-electron reduction of pyrimidine dimers significantly decreases the activation energy for splitting via nonsynchronous concerted or fully nonconcerted (i.e., stepwise) pathways but not via a fully concerted pathway. The pathway in which a–a′ cyclobutyl bond cleavage (Chart I) is advanced over b–b′ bond cleavage is most accelerated by one-electron reduction of the dimer. The transfer of an electron to the dimer has, in effect, facilitated dimer cycloreversion by providing stabilization to the system as it splits, thereby offsetting the energy requirements of σ-bond breaking.

The above semiquantitative treatment provides a plausible explanation for why dimer radical anions split more readily than their neutral precursors, thereby offering insights into why at least one photolyase first reduces its substrate to initiate the repair of photodamage in DNA.

Acknowledgment. Grateful acknowledgment is made to the donors of The Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We thank Dr. R. Nieman for assistance with the NMR kinetics automation program. The NMR studies were facilitated by a chemical instrumentation grant from NSF (CHE-8409644).

(20) A similar treatment has been applied to radical cations: Bauld, N. L.; Bellville, D. J.; Pabon, R.; Chelsky, R.; Green, G. *J. Am. Chem. Soc.* **1983**, *105*, 2378–2382.