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Model studies of the (6-4) photoproduct photoreactivation: synthesis and photosensitized splitting of uracil oxetane adducts

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Abstract

Uracil oxetane adducts, which are model compounds for the oxetane intermediates generated during the formation of (6-4) photoproducts or in their photoenzymatic repair, have been synthesized using 1,3-dimethyluracil with carbonyl compounds. On the basis of fluorescence measurements and photolysis experiments, it is demonstrated that the oxetane adducts can be split into the nucleotide base and carbonyl compounds via an electron transfer reaction from photosensitizer. The reaction is more efficient for a stronger electron donor.
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Keywords: (6-4) Photoproducts; Oxetane intermediate; DNA photorepair; Electron transfer

1. Introduction

DNA is damaged by ultraviolet component from sunlight to give a variety of potentially mutagenic photoproducts [1–4]. The pyrimidine cyclobutane dimers are the most abundant photoproducts among the major three types of photoinduced DNA damage. For this reason, most studies of chemical and biochemical aspects of DNA photodamage have focused on this class of photoproducts. However, more recent

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Oxetane intermediates   (6-4) Photoproducts
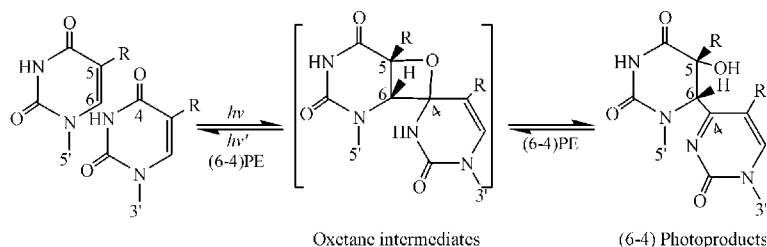
R = H or CH$_3$

(6-4)PE = (6-4)Photolyase enzyme

Scheme 1.

work has suggested that some of the less abundant UV photoproducts might actually be more effective at causing damaging mutations [5,6].

The pyrimidine(6-4)pyrimidone adducts, simply termed (6-4) photoproducts, constitute a second class of stable photoproducts in DNA and also play a role in UV-induced cytotoxic damage [1,7–11]. Formation of a (6-4) product from T*+T in DNA and U*+U in RNA via an initial Paterno-Büchi cycloaddition to form an oxetane intermediate is depicted in Scheme 1. Clivio et al. [12] have reported the isolation of a thietane intermediate generated during the formation of a (6-4) photoproduct involving 4-thiothymidine. The work is one of the few pieces of experimental evidence supporting the proposed mechanism for the formation of a (6-4) photoproducts. In 1993, Todo et al. [13] reported the discovery of an enzyme from *Drosophila melanogaster* cell extracts, which can mediate the reversal of (6-4) photoproducts under UVA and blue light. The (6-4) photolyase enzymes with similar function and sequence were subsequently discovered in other species [14–16]. Sancar and co-workers [17–19] suggested an electron transfer (ET) repair mechanism for the (6-4) photolyase, which is analogous to that of cyclobutane pyrimidine dimer (CPD)[1] photolyase enzyme [20–23]. DNA (6-4) photoproduct photolyase can specifically bind to damaged sites containing a pyrimidine(6-4)pyrimidone and cause it to revert back to the oxetane or azetidine (in the case of cytosine) intermediate. This is followed by the absorption of a UV-A or visible light photon, resulting in an electron transfer from reduced flavin to the intermediate. The radical anion of the oxetane or azetidine fragment rapidly and back electron transfer restores the two pyrimidine bases and the functional form of flavin ready for a new cycle of catalysis (Scheme 1) [17–19,24].

Rapid fragmentation of the oxetane radical anion is a key premise of this mechanism. At the time of the original proposal there was no definitive evidence suggest-

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[1] *Abbreviations used:* CPD, cyclobutane pyrimidine dimer; DMA, *N,N*-dimethylaniline; DMT, 1,3-dimethylthymine; DMU, 1,3-dimethyluracil; DMT, 1,3-dimethylthymine; ET, electron transfer; MHA, 10-methyl-9,10-dihydroacridine.

ing that any oxetane adduct of a pyrimidine would fragment following one-electron transfer because the natural oxetane intermediates are not isolable, as they spontaneously undergo thermal ring-opening reaction to give the observed (6-4) photoproducts (see Scheme 1). Thymine oxetane adduct with benzophenone has been obtained in early research [25]. Utilizing synthesized model thymine-based oxetane adducts with aromatic carbonyl compounds, Falvey and co-workers [26,27] demonstrated that both sensitized and direct irradiation of these model compounds resulted in their splitting to give the base and the corresponding carbonyl compound. These experiments provided evidence for the ET mechanism.

Gurzadyan and Görner [28] demonstrated the formation of (6-4) photoproducts in simple 5'-uracil- and 5'-cytosine-containing pyrimidine dinucleotides including UpU, CpU, and CpT. In a more recent work, Douki and Cadet [29] found that (6-4) photoproducts can form at 5'-thymine–cytosine and cytosine–cytosine sites in DNA by LC/MS analysis. Moreover, it is known that the 5'-cytosine residues in the (6-4) photoproducts can be deaminated to form uracil-containing (6-4) photoproducts. Therefore, it would be of interest to synthesize uracil oxetane adducts and examine whether these compounds show a similar ET repair mechanism. In this work, we prepared oxetane adducts of 1,3-dimethyluracil (DMU) with benzophenone and benzaldehyde, and investigated their behavior under electron transfer conditions by fluorescence measurements and photolysis experiments. In addition, we carried out comparison of DMU and 1,3-dimethylthymine (DMT) from [2 + 2] photocycloaddition with carboxyl compounds and the splitting reaction of their formed oxetanes.

2. Experimental details

2.1. General methods

^{1}H, ^{13}C NMR, and PS-NOESY spectra were measured on a Bruker AV 400 spectrometer operating at 400.13 MHz for ^{1}H and 100 MHz for ^{13}C. Mass spectra were obtained on a Micromass GCF TOF mass spectrometer. UV–Vis spectra were measured on a Lambda Bio20 UV/VIS spectrometer using a 10 mm path length quartz cell. Fluorescence emission spectra were measured on a 970CRT fluorescence spectrometer (Shanghai Analysis Instrument). The quenching constant ($\tau_s k_q$) of the sensitizer by DMU-base oxetanes was measured through Stern–Volmer analyses for each oxetane/sensitizer.

Photolysis experiments were performed by a series of turntable experiments. In these experiments, a group of samples are placed on a rotating turntable. The Hg lamp is fixed in the center of the turntable. Using this procedure, the samples are all irradiated under similar conditions. Each sample was prepared in 2 ml spectroscopic grade CH_3CN solution containing 2 mM sensitizer and 2 mM oxetane. The solution was sealed in a Pyrex (>290 nm) tube, deaerated by high purity nitrogen bubbling for 20 min, and irradiated for 10 min with a 300 W high pressure Hg lamp. Deviations from the above mentioned conditions are indicated in the text. The

photolysis mixtures were analyzed using a high performance liquid chromatography (HPLC) instrument (HP Agilent 1100 series) with an UV/Vis absorption detector and a C-8 reverse-phase column and methanol–water (60:40) as the eluent. The flow rate in all cases was 1 ml/min and column temperature was 30 °C. The products and reactants were identified by co-injections, separated, and their peak areas quantified.

2.2. Chemicals

Aniline, *p*-toluidine, *N*,*N*-dimethylaniline (DMA), 1-naphthylamine, and phenanthrene are commercially available and were used as received after being recrystallized or redistilled twice. Uracil, benzophenone, benzaldehyde, and acridine are also commercially available. Acetonitrile (for HPLC) were used as received. 10-Methyl-9,10-dihydroacridine (MHA) was prepared from 10-methylacridium iodide by reduction with $NaBH_4$ in methanol and purified by recrystallization from ethanol. 10-Methylacridium iodide was prepared by the reaction of acridine (Merck, Darmstadt, FR Germany) with methyl iodide in acetone [30].

2.3. Preparation of Z-2,4-dimethyl-8,8-diphenyl-7-oxa-2,4-diazabicyclo[4.2.0]octane-3,5-dione

1,3-Dimethyluracil (DMU) was prepared by methylation of uracil with methyl sulfate in alkaline aqueous solution [31] (Scheme 2). Benzophenone (2.6 g, 14 mmol) was added to DMU (1.0 g, 7 mmol) in 70 ml CH_3CN. The solution was placed in a pyrex reactor, bubbled with N_2 to saturate, and irradiated with a 300 W high pressure Hg lamp for 8 h. The solvent was removed by rotary evaporation. Elution of the residue over silica with 4:1 petroleum ether/EtOAc gave 0.34 g of **1** as white crystals, yield 14.8%, mp 134–135 °C; ^{1}H NMR (DMSO, 400 MHz) δ 2.81 (s, 3H), 2.92 (s, 3H), 5.12 (d, $J = 8.8$ Hz, 1H), 5.19 (d, $J = 8.8$ Hz, 1H), 7.33 (m, 10H); ^{13}C NMR (DMSO, 100 MHz) δ 167.11, 151.22, 143.64, 139.62, 128.44, 128.06, 127.80, 125.44, 125.23, 95.27, 70.78, 59.28, 34.94, 26.75; IR 1717, 1682 cm^{-1}; TOFMS (EI) Calcd. for (M$^+$) $C_{19}H_{18}N_2O_3$: 322.1317. Found: 322.1343.
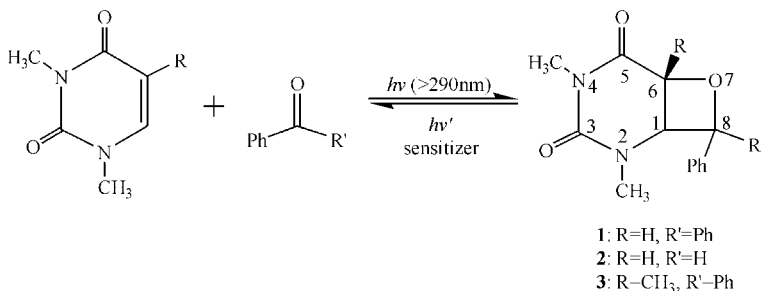
Scheme 2.

2.4. Preparation of 2.4 (6Z,8Z)-2,4-dimethyl-8-phenyl-7-oxa-2,4-diazabicyclo [4.2.0]octane-3,5-dione

Compound (**2**) was obtained by following the procedure described above using DMU (0.7 g, 5 mmol) and benzaldehyde (1.06 g, 10 mmol). This gave 0.13 g of **2**, yield 10.6%, mp 104–106 °C; ^{1}H NMR (DMSO, 400 MHz) δ 2.85 (s, 3H), 3.13 (s, 3H), 4.33 (dd, $J_1 = 5.6$ Hz, $J_2 = 8.8$ Hz, 1H), 5.23 (d, $J = 8.8$ Hz, 1H), 5.73 (d, $J = 5.6$ Hz, 1H), 7.43 (m, 5H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 167.03, 151.69, 138.98, 129.23, 129.18, 125.19, 91.15, 71.74, 58.70, 34.33, 28.11; IR 1715, 1665 cm^{-1}; TOFMS (EI) Calcd. for (M$^+$) C$_{13}$H$_{14}$N$_2$O$_3$: 246.1004. Found: 246.1014. The PS-NO-ESY spectrum of **2** in CDCl$_3$ (see Fig. 1) shows signals of correlation between the 6H(δ 5.21), 8H(δ 5.64), and 1H(δ 4.23), respectively. It indicates that the three protons, 6H, 1H, and 8H are at the same side of oxetane ring.
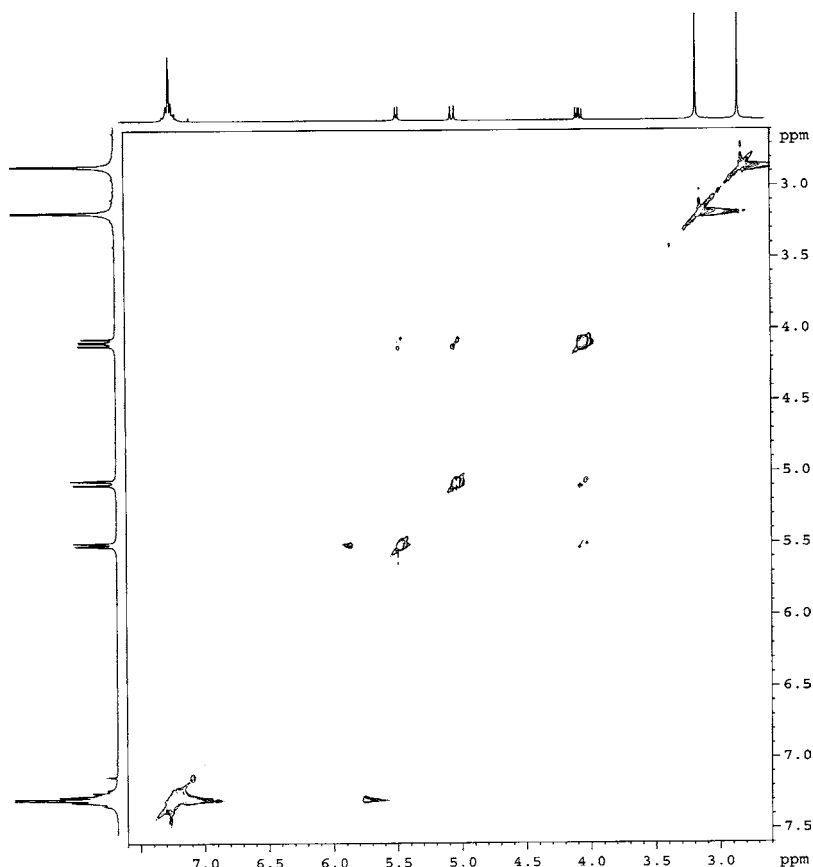

Fig. 1. The PS-NOESY spectrum of **2** in CDCl$_3$. Here have signals of correlation between the 6H(δ 5.21), 8H(δ 5.64), and 1H(δ 4.23), respectively.

3. Results and discussion

3.1. Fluorescence quenching experiments

The excitation wavelength of the sensitizer was chosen to ensure that none of the light was absorbed by the oxetane, and fluorescence intensity obtained from its fluorescence peak. Both oxetane **1** and **2** would quench fluorescence of some electron-rich compounds, so fluorescence quenching experiments were performed. Solutions of the sensitizers were prepared in spectroscopic grade CH_3CN, and their fluorescence intensities were measured with varying concentrations of added oxetane. In each case the fluorescence intensity decreased as oxetane was added. The results of the fluorescence scans were analyzed by the Stern–Volmer equation, $(F_0 - F)/F = \tau_s k_q [Q]$. The $\tau_s k_q$ values were obtained from the slopes of Stern–Volmer plots. One example is shown in Fig. 2, which is generated by the fluorescence quenching of MHA by oxetane **1**. Using literature values [32] for the lifetimes of fluorescence (τ_s), the quenching rate constants (k_q) were obtained and listed in Table 1. These values are at or near the diffusion limit, ranging from 0.46 to $27 \times 10^9 \, M^{-1} \, s^{-1}$. We note that the strongest excited state electron donor, DMA, gives the highest k_q values for **1**.

3.2. The photolysis experiments

We measured product distribution of each case by HPLC and the results are listed in Table 1. The photosensitizers in Table 1 are able to photosensitize the splitting of oxetanes. The most efficient splitting reaction is seen in the presence of the strongest electron donor DMA. Direct irradiation without a sensitizer also leads to splitting of oxetanes. Besides the splitting reaction, few side products were also observed. Because DMU and benzophenone/benzaldehyde are unstable under the photolysis condition, the side products would result from secondary photolysis of the substrates.
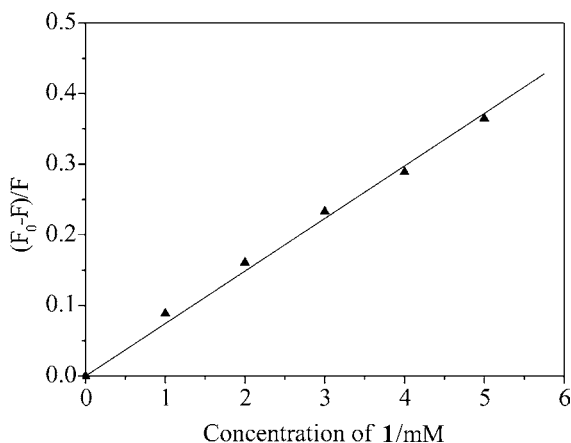

Fig. 2. Stern–Volmer analysis of the fluorescence quenching of MHA by oxetane **1** (λ_{ex} at 300 nm, fluorescence intensity obtained from its fluorescence peak 365 nm).

Table 1
Splitting reaction of oxetanes **1** and **2** in the presence of various photosensitizers[a]

Sensitizer	E_{ox}/V SCE in CH_3CN	Reactant oxetane	E_{ox}^*/V SCE	Yield %			$k_q\tau_s(k_q)$ (M^{-1} (10^9 M^{-1} s^{-1}))
				Conversion	PhC(O)R	DMU	
None		**1**[c]		7	55	52	
		1[d]		18	86	89	
Photosensitizers							
Aniline	+0.98[b]	**1**	−2.77	12	56	52	92(5.5)
p-Toluidine	+0.78[b]	**1**	−2.86	21	50	64	53(11)
DMA	+0.53[b]	**1**	−3.00	33	25	65	79(27)
		2		20	45	78	256(85)
MHA	+0.81[e]	**1**	−2.65	16	52	45	74(10)
1-Naphthylamine	+0.54[b]	**1**	−2.46	20	18	44	69(3.5)
Phenanthrene	+1.50[b]	**1**	−2.07	8	<6	6	28(0.46)

[a] Sensitizer and oxetane were prepared as 2 ml, N_2-saturated CH_3CN solution in a Pyrex (>290 nm) tube, respectively, unless otherwise indicated. Samples were irradiated for 10 min with a 300 W high pressure Hg lamp.

[b] From [32].

[c] Direct photolysis without sensitizer carried out in a Pyrex tube (>290 nm, 4 mM oxetane **1**) irradiated 60 min, in a quartz tube (2 mM oxetane **1**) irradiated 10 min, respectively.

[d] Direct photolysis without sensitizer carried out in a Pyrex tube (>290 nm, 4 mM oxetane **1**) irradiated 60 min, in a quartz tube (2 mM oxetane **1**) irradiated 10 min, respectively.

[e] From [30].

This is also reason why the yields of benzophenone and DMU in sensitized photolysis reaction with DMA and 1-naphthylamine are unequal.
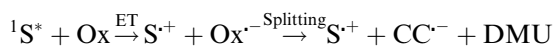
On the basis of energetic grounds, energy transfer mechanisms can be excluded in the splitting reactions. Both **1** and **2** are transparent under light at wavelength more than 300 nm, and we can estimate the singlet energies of oxetanes **1** and **2** at more than 399 kJ mol^{-1}. The excited singlet energies of the sensitizers are not more than 362 kJ mol^{-1} (for aniline). In addition, the acceptor electron site should be the uracil moiety of the oxetanes. This is based on the likely reduction potentials of functional groups presented in **1** and **2**. The uracil residue of the oxetane has a saturated 5,6 bond and can be considered analogous to the DMU cyclobutane dimer with a reduction potential of ca. −2.3 V (vs SCE). The unconjugated benzene ring(s) on the ketone/aldehyde portion of the oxetane would be much more difficult to reduce as benzene has a reduction potential of ca. −3.2 V [33]. Therefore, the pyrimidine ring is the most energetically favorable location for single-electron reduction [26].

3.3. Calculation of free energy changes of electron transfer reactions from excited singlet sensitizers to the oxetane

The free energy changes (ΔG) of the putative electron transfer reactions from excited singlet sensitizers to oxetane **1** were calculated according to Rehm–Weller equation [34]:

$$\Delta G \text{ (kJ mol}^{-1}) = 96.5[E_{ox}(D) - E_{red}(A) - e_0^2/\varepsilon R_{D^+A^-}] - \Delta E_{0,0}, \quad (1)$$

where $E_{ox}(D)$ and $E_{red}(A)$ are half-wave potentials of electron donors and acceptors, respectively. $\Delta E_{0,0}$ is the lowest energy level of excited singlet sensitizer obtained from their fluorescence spectroscopy. The $-e_0^2/\varepsilon R_{D^+A^-}$ is the solvation energy of an ion pair D^+A^-. It is $-0.06\,V$ in acetonitrile [35]. In the case of the same oxetane, $E_{red}(A)$ and $e_0^2/\varepsilon R_{D^+A^-}$ are constant, $(E_{ox} - \Delta E_{0,0}/96.5)$ is its excited state oxidation potential, E_{ox}^*/V. E_{ox}^* is obtained from literature values for the oxidation potential (E_{ox}) and the singlet state energy, $\Delta E_{0,0}$ from its fluorescence spectra. Calculated E_{ox}^* are listed in Table 1. As E_{ox}^* becomes increasingly negative, k_q increases. From this result, a qualitative generalization can be made. The correlation of k_q and E_{ox}^* is consistent with the electron transfer mechanism as follows. Singlet excited state of sensitizer ($^1S^*$) transfers an electron to the oxetane (Ox) to give sensitizer radical cation ($S^{\cdot+}$) and oxetane radical anion ($Ox^{\cdot-}$). The oxetane anion would rapidly split to form DMU and carbonyl compound radical anion ($CC^{\cdot-}$). In the case of 1,3-dimethyl-thymine-derived oxetane anion, the splitting process was $>5 \times 10^7\,s^{-1}$ [27]:

$$S \xrightarrow{h\nu} {}^1S^*$$

$$^1S^* + Ox \xrightarrow{ET} S^{\cdot+} + Ox^{\cdot-} \xrightarrow{Splitting} S^{\cdot+} + CC^{\cdot-} + DMU$$

3.4. Uracil and thymine oxetane: a comparison of the synthesis and photosensitized splitting

Oxetane **3** was synthesized by the [2 + 2] photocycloaddition of 1,3-dimethylthymine (DMT) and benzophenone according to the procedure used for the synthesis of **1** and **2**. We have found that the formation of DMT-based oxetane is faster (for example **3**, irradiation for only 5 h) and the yields (**3**, 40%) are higher than those of DMU-based oxetanes. The same results were obtained from repeating the synthesis many times.

Photocycloadditions of pyrimidine with aromatic carbonyl compounds, which are Paternò–Büchi reactions, are generally considered to proceed through a diradical intermediate [36]. In the reaction process of pyrimidine and aromatic carboxyl compounds, the carboxyl compounds were excited to form singlet excited state, then transited to triplet excite state ($^3CC^*$) via intersystem crossing. It is well known that this is the n–π* transition, and electron density for O atom of carboxyl group would decrease. Hence, O atom of $^3CC^*$, as an electrophilic center, attacks C6 of pyrimidine to produce a diradical intermediate **4**. So the first step in the photocycloaddition between $^3CC^*$ and pyrimidine is an electrophilic reaction. Generally, O atom of triplet carbonyl compound tending to attack the more electron-rich carbon among C=C of enamine [37]. Using Gaussian 98 program [38], we calculated to obtain the electron densities at C6 of DMT and DMU, 1.32 and 1.18, respectively. Therefore, O atom of $^3CC^*$ more easily attack C6 of DMT than DMU. In addition, stability of the diradical intermediate **4** is also a possible important factor. The result can be used to explain why the formation of thymine-based (6-4) photoproducts in DNA under UV light occurs more easily.

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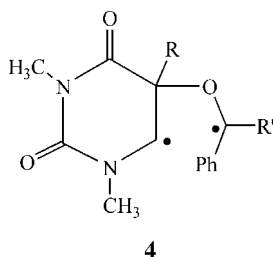
However, the rates of the photosensitized splitting of DMU oxetanes are faster than those of DMT oxetanes [26,27]. The $k_q\tau_s$ of aniline is 91 M^{-1} for DMU oxetane **1**, and 81 M^{-1} for DMT oxetane **3** [26]. In the case of DMA, the k_q for **3** is 1.79×10^{10} M^{-1} s^{-1} [27], and 2.7×10^{10} M^{-1} s^{-1} for **1**. Splitting of the oxetane by accepting an electron produces an oxetane anion, and the property of the oxetane anion would be an important factor to determine splitting reaction rate of oxetane. The detailed mechanism warrants further investigation.

4. Summary

The photochemical behavior of the DMU-derived oxetanes is in many ways similar to that of the oxetane intermediates in the putative photoreactivating process of (6-4) photoproduct by the DNA photolyase. Splitting of the oxetanes can be promoted either by direct irradiation or by sensitization with electron donors. It appears that cycloreversion is a general behavior of pyrimidine oxetane anion radicals. The reactions proceed clearly with a number of substrates and give side products in small quantities due to secondary photolysis. The results provide further support for the ET repair mechanism involving oxetane intermediates. The electron-transfer mechanism is attractive because the (6-4) product photolyase and CPD photolyases show sequence identity suggesting a common mechanism. In addition, the active form of the catalytic cofactor in both photolyases is a fully reduced flavin.

Acknowledgments

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