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Oxidative Cleavage of a Cyclobutane Pyrimidine Dimer by Photochemically Generated Nitrate Radicals ($NO_3^•$)

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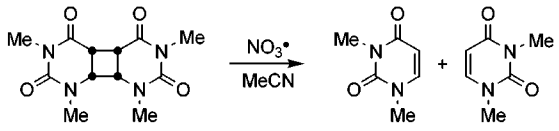
ABSTRACT



1a **2**

Photochemically generated nitrate radicals ($NO_3^•$) cleave the stereoisomeric *N*,*N*-dimethyl-substituted uracil cyclobutane dimers 1a–d into the monomeric uracil derivative 2 as the major reaction pathway. A preferred splitting of the *syn* dimers 1a,b was observed. The reaction is expected to proceed through initial one-electron oxidation with formation of an intermediate cyclobutane radical cation 11. In addition to cycloreversion, competing reaction steps of 11, which lead to the observed byproducts, are suggested.

The thymine cyclobutane dimer (T<>T) is the most important product formed between two adjacent thymine bases through a [2 + 2] photocycloaddition upon exposure of the DNA to UV radiation. This damage can lead, if not repaired, to mutations or cancerogenous cell growth.[1] The repair of T<>T in bacterials is achieved by the dimer-specific repair enzyme photolyase through a direct one-electron reduction followed by reversion of the resulting unstable cyclobutane radical anion and subsequent oxidation to the thymine monomers.[2] It was shown that the cleavage of the cyclobutane pyrimidine dimer could also proceed through an oxidative pathway, since the cyclobutane radical cation is similarly unstable.[3] This oxidative repair was recently used by Barton's group as a mechanistic probe to examine the long-range charge transfer through the DNA base stack.[4]

During the course of our ongoing study on the oxidative damage of nucleosides caused by nitrate radicals ($NO_3^•$),[5,6] we wondered whether the oxidation power of the electrophilic $NO_3^•$ could also have a positive impact on DNA by repairing already existent damages through an oxidative pathway, e.g., pyrimidine cyclobutane dimers. The oxidative cleavage of the *cis-syn* cyclobutane thymine dimer and the stereoisomeric C5−C5′ linked thymine dimers by other electrophilic radicals, e.g., the hydroxyl radical ($OH^•$) and the sulfate radical anion ($SO_4^{•-}$), respectively, has been described in recent literature, and both a one-electron oxidation as well as a hydrogen atom abstraction has been

(1) (a) Friedberg, E. C.; Walker, G. C.; Siede, W. *DNA Repair and Mutagenesis*; American Society for Microbiology: Washington, DC, 1995. (b) Taylor, J.-S. *Acc. Chem. Res*. **1994**, *27*, 76. (c) Begley, T. P. *Acc. Chem. Res*. **1994**, *27*, 394.

(2) See, for example: (a) Park, H. W.; Kim, S.-T.; Sancar, A.; Deisenhofer, J. *Science* **1995**, *268*, 1866. (b) Kim, S.-T.; Volk, M.; Rousseau, G.; Heelis, P. F.; Sancar, A.; Michel-Beyerle, M.-E. *J. Am. Chem. Soc.* **1994**, *116*, 3115. (c) Heelis, P. F.; Kim, S.-T.; Okamura, T.; Sancar, A. *J. Photochem. Photobiol. B* **1993**, *17*, 219. (d) Butenandt, J.; Eker, A. P. M.; Carell, T. *Chem. Eur. J*. **1998**, *4*, 642.

(3) Voityuk, A. A.; Michel-Beyerle, M.-E.; Rösch, N. *J. Am. Chem. Soc*. **1996**, *118*, 9750.

(4) (a) Vicic, D. A.; Odom, D. T.; Núñez, M. E.; Gianolio, D. A.; McLaughlin, L. W.; Barton, J. K. *J. Am. Chem. Soc*. **1999**, *122*, 8603. (b) Dandliker, P. J.; Homlin, R. E.; Barton, J. K. *Science* **1997**, *275*, 1465. (c) Dandliker, P. J.; Núñez, M. E.; Barton, J. K. *Biochemistry* **1998**, *37*, 6491.

(5) Nitrate radicals are the most important oxidants in the nighttime atmosphere: Wayne, R. P.; Barnes, I.; Biggs, P.; Burrows, J. P.; Canosa-Mas, C. E.; Hjorth, J.; Le Bras, G.; Moortgat, G. K.; Perner, D.; Restelli, G.; Sidebottom, H. The Nitrate Radical: Physics, Chemistry and the Atmosphere, Wayne, R. P., Ed.; *Atmos. Environ., Part A* **1991**, *25*.

(6) Wille, U.; Steenken, S., manuscript in preparation.

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suggested as the primary reaction step.[7] As a starting point in our study on the $NO_3^\bullet$ reaction with pyrimidine cyclobutane dimers, we decided to use simple model compounds, the stereoisomeric C5−C5′ and C6−C6′ linked *N*-methylated dihydrouracil dimers **1a**−**d** (Figure 1).[8]

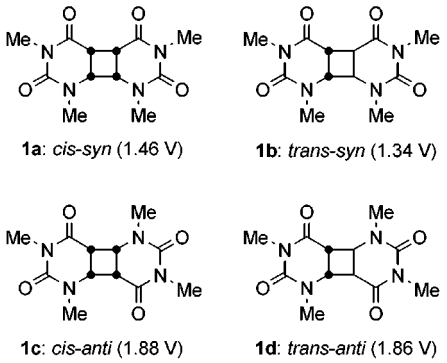


Figure 1. Stereoisomeric uracil cyclobutane dimers **1a**−**d** and their irreversible half-peak anodic potentials $E_{p/2}$ in MeCN (in parentheses) according to ref 9.

$NO_3^\bullet$ was in situ generated in an acetonitrile solution of **1a**−**d** through photolysis of cerium(IV) ammonium nitrate (CAN) according to[10]

$$(NH_4)_2Ce(NO_3)_6 + h\nu \rightarrow NO_3^\bullet + (NH_4)_2Ce(NO_3)_5$$

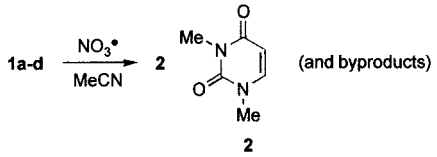
These conditions could be considered to simulate the hydrophobic environment in the DNA base stack. The product analysis was performed by GC and GC−MS.[11] The dimers **1a**−**d** were taken as excess component with [**1**]:[CAN] = 5 to avoid a significant consumption of the primary reaction products by $NO_3^\bullet$. The experimental results are compiled in Table 1.[12] Reaction of $NO_3^\bullet$ with a mixture of **1a**−**d**, which contained each isomer in approximately equal amounts, led to formation of the monomer **2** according to the general reaction shown in Scheme 1 (entry 1).[13] Interestingly, the rate of the $NO_3^\bullet$-induced splitting showed a significant dependence on the stereochemistry and the substitution pattern at the cyclobutane ring in **1a**−**d**. It appears that the *syn* isomers **1a** and **1b** are cleaved faster than the *anti* isomers **1c** and **1d**.

Table 1. GC Analysis of the Reaction of the Uracil Cyclobutane Dimers **1a**−**d** with $NO_3^\bullet$ [a]

	sample composition	
entry	before reaction (%)[b]	after reaction (%)[b]
1	**1a** (21); **1b** (26); **1c** (28); **1d** (25)	**1a** (<1); **1b** (1); **1c** (24); **1d** (18); **2** (25); **3** (2): **4** (16); **5** (4); **6** (2); **7** (5); n.i.[c] (2)
2	**1a** (100)	**1a** (7); **2** (50); **4** (33); **5** (5); **7** (5)
3	**1b** (100)	**1b** (37); **2** (14); **3** (4); **4** (35); **5** (7); **7** (3)
4	**1c** (100)	**1c** (39); **2** (33); **7** (3); **8** (10); **9** (1); n.i. (14)
5	**1d** (100)	**1d** (30); **2** (34); **6** (15); **7** (9); **8** (11); **9** (1)
6	**1a** (67); **10** (33)[d]	**1a** (13); **10** (26); **2** (33); **4** (14); **5** (2); **7** (3); n.i. (9)[e]
7	**1c** (66); **10** (34)[d]	**1c** (46); **10** (8); **2** (33); **7** (2); **8** (2); n.i. (9) [e]

[a] [**1**]:[CAN] = 5. [b] Relative peak area. [c] n.i. = not identified. [d] [**1**]:[**10**]:[CAN] = 1:1:0.5. [e] Product formed in the reaction of **10** with $NO_3^\bullet$.

Scheme 1



2

To confirm the role of $NO_3^\bullet$ in the cleavage process, the dimer mixture **1a**−**d** was also treated with CAN without irradiation (data not shown). Only a very small amount of

(7) (a) Ito, T.; Shinohara, H.; Hatta, H.; Nishimoto, S.; Fujita, S. *J. Phys. Chem. A* **1999**, *103*, 8413. (b) Heelis, P. F.; Deeble, D. J.; Kim, S.-T.; Sancar, A. *Int. J. Radiat. Biol*. **1992**, *62*, 137.

(8) The dimers **1a**−**d** were prepared in analogy to ref 2d and separated by column chromatography. The stereochemistry at the cyclobutane ring was assigned according to: Fahr, E.; Maul, P.; Lehner, K.-A.; Scheutzow, D. *Z. Naturforsch*. **1972**, *27b*, 1481.

(9) Pac, C.; Kubo, J.; Majima, T.; Sakurai, H. *Photochem. Photobiol*. **1982**, *36*, 273.

(10) (a) Lietzau, L.; Wille, U. *Heterocycles* **2001**, *55*, 377. (b) Baciocchi, E.; Del Giacco, T.; Murgia, S. M.; Sebastiani, G. V. *J. Chem. Soc., Chem. Commun*. **1987**, 1246 and literature cited therein.

(11) **Typical Experimental Procedure.** In a duran reactor 25 μmol of the dimer (or the dimer mixture) and 5 μmol of CAN were dissolved in 6 mL of acetonitrile and irradiated under argon for 2 h using a medium-pressure mercury lamp. A sample of the reaction mixture was filtrated (SiO_2, ethyl acetate) and analyzed by GC. GC: Varian 3400cx; column SE 30, 50 m; temperature program $120_5 \rightarrow 250_{22}$, heating rate 10 °C min^{-1}. GC-MS: Finnigan MAT MS TSQ 70; Varian 3400; column SE 30, 30 m; temperature program $80_2 \rightarrow 280$, heating rate 15 °C min^{-1}.

(12) The GC data given in Table 1 are relative peak areas determined without an internal standard. For each of the components in this investigation the response factor in the GC is approximately the same. In addition, it was verified by an independent experiment that the organic material could be quantitatively recovered after the irradiation and workup procedure (see ref 11).

(13) It was verified that no photoinduced splitting of **1a**−**d** occurred in the absence of CAN.

(14) Ce^{4+}/Ce^{3+}: $E° = 1.61$ V vs NHE; *Handbook of Chemistry and Physics*, 63rd ed.; CRC Press, Boca Raton, Florida, 1995; D-162.

(15) Compounds **3**, **5**, and **6** are not described in the literature. As a result of the fragmentation of the cyclobutane unit in the electron impact ion source of the MS, the appearance of a peak at *m*/*z* 140 in the EI spectra, which corresponds to a fragment structure of the monomer **2**, was taken as an indication whether the cyclobutane ring was destroyed or at least modified. However, the structural assignments of **5** and **6** are still very speculative. Compound **5** may be deduced from the dimer **1** by formal loss of a [NCH] fragment. EI: *m*/*z* (%) 253 (35) [M^+], 221 (10), 168 (45), 140 (100), 83 (25), 42 (30). Compound **6** may be deduced from **1** by formal loss of a [NH_2CH_3CO] fragment. The fact that the molecular peak is also the base peak was taken as an indication for the stability of the molecule, leading to the proposed rearranged structure. EI: *m*/*z* (%) 221 (100) [M^+], 193 (15), 164 (10), 136 (20), 108 (20), 82 (15), 42 (10).

the monomer **2** was formed even after a reaction time of 2.5 days. Since CAN is known to be a strong one-electron oxidant,[14] we suggest that **2** is produced through a slow electron-transfer process from **1**.

The data in Table 1 show that cleavage to the monomer **2** was not the exclusive reaction; formation of varying amounts of byproducts **3**−**9** was also observed. The origin of these compounds was determined by reacting **1a**−**d** separately with $NO_3^{\bullet}$, and their structures were tentatively assigned by GC−MS (Figure 2 and Table 1, entries 2−5).[15]

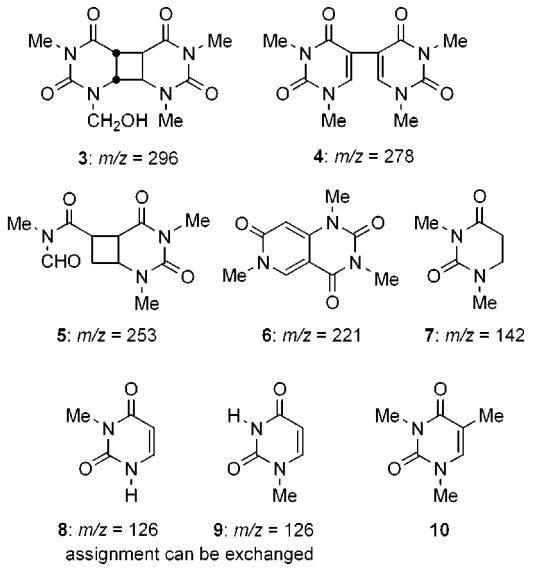
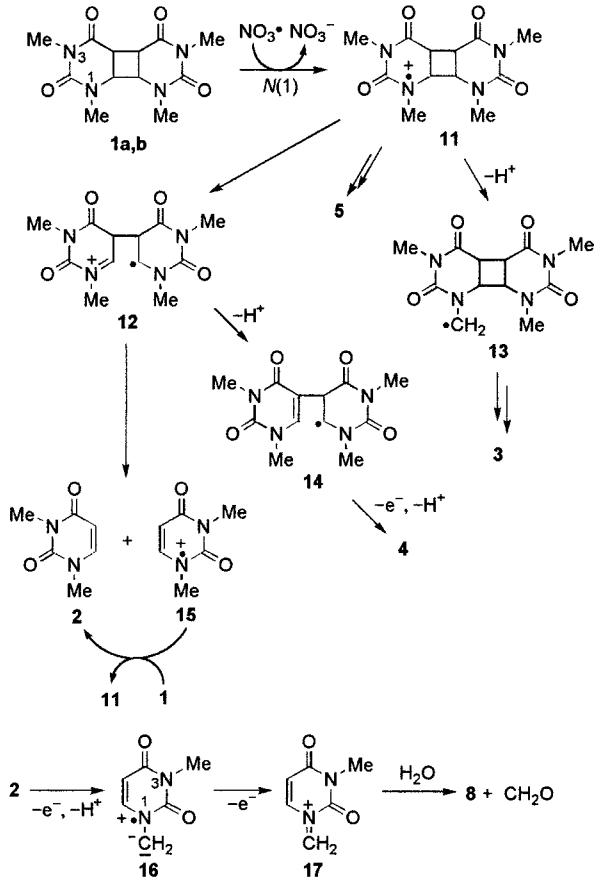


Figure 2.

Interestingly, with the exception of the dihydrouracil **7**, which is formed in a reaction of the monomer **2** with $NO_3^{\bullet}$ and therefore appeared in each reaction,[16] the product distribution strongly depended on the stereochemistry at the cyclobutane ring in **1a**−**d**. The C5−C5′ linked uracil dimer **4**, which is the most important byproduct, and the formamide **5** were only formed in the reaction of the *syn* isomers **1a** and **1b**. However, whereas in the case of **1a** a preferred splitting to the monomer **2** and minor formation of **4** was observed, the latter is the major product in the reaction of $NO_3^{\bullet}$ with **1b**, and cleavage into **2** is only a minor reaction pathway.[17] The $NO_3^{\bullet}$ reaction with **1b** lead also to formation of the hydroxylated species **3**. The *N*-dealkylated uracils **8** and **9**, as well as compound **6**, exclusively appeared, although in only minor amounts, in the $NO_3^{\bullet}$ reaction of the *anti* isomers **1c** and **1d**, which are, like **1a**, cleaved to the monomer **2** as the major reaction pathway.

Both electron transfer and hydrogen atom abstraction by $NO_3^{\bullet}$ may be considered as the primary step in the reaction with **1a**−**d**.[5] Competition experiments, where equal amounts of *N*(1),*N*(3)-dimethylthymine (**10**) and the dimer **1a** and **1c**, respectively, were irradiated in the presence of 0.5 equiv of CAN, showed that **1a** is more reactive than **1c** and even than **10** (Table 1, entries 6 and 7).[18,19] Since the reaction of **10** with $NO_3^{\bullet}$ is expected to proceed through an initial electron transfer,[6] in addition to the finding of a slow CAN-induced oxidative cleavage of **1**, we believe that this holds true also for the $NO_3^{\bullet}$-induced splitting of the dimers **1a**−**d**, in particular, as the oxidation potentials of **1a**−**d** are accessible by $NO_3^{\bullet}$ (see Scheme 1).[20] On the basis of this, a mechanism could be proposed, which is shown for the reaction of $NO_3^{\bullet}$ with the *syn* isomers **1a**,**b** in Scheme 2. Initial electron

Scheme 2



transfer at N(1) adjacent to the cyclobutane ring would lead to the radical cation **11**.[21] This species could loose an

(16) The mechanism for formation of **7** is not clear. The one-electron reduction of **2** requires a potential of −2.11 V in MeCN: Scannell, M. P.; Prakash, G.; Falvey, D. E. *J. Phys. Chem. A* **1997**, *101*, 4332. Yet, we were not able to determine the reductive species in our system, as both Ce(III) and the nitrate ion could be strictly excluded.

(17) Formation of compound **4** by photosensitized oxidative splitting from the *syn* uracil cyclobutane dimers **1a**,**b** was reported in the literature: Elad, D.; Rosenthal, I.; Sasson, S. *J. Chem. Soc. C* **1971**, 2053.

(18) In these cases, besides the known compounds arising from the reaction of **1a,c** with $NO_3^{\bullet}$, product signals originating from a reaction of $NO_3^{\bullet}$ with **10** also appeared but were not identified.

(19) This may be important with respect to the fact that the T<>T lesions in DNA possess a *cis-syn* configuration at the cyclobutane ring. A preferred photochemical splitting of the *syn* configurated uracil cyclobutane dimers was described in ref 17. An analogous behavior was observed in splitting experiments using a photosensitizer and was explained by the lower oxidation potentials of the *syn* cyclobutane dimers: Rosenthal, I.; Rao, M. M.; Salomon, J. *Biochem. Biophys. Acta* **1975**, *378*, 165; and ref 9.

aminomethyl unit to give the formamide **5**. Deprotonation at the *N*(1)-methyl group in **11** would lead to radical **13**, which could be expected to be the precursor for the hydroxylated species **3** through further reactions with $NO_3^{\bullet}$. Formation of the monomer **2** is believed to proceed stepwise from **11** by first splitting of the C6−C6′ bond to give **12**, followed by cleavage into **2** and its radical cation **15**.[22] The latter could initiate a radical chain through oxidation of further dimer molecules **1**.

The suggestion of a radical chain in the present system is based on the finding that consumption of **1a**−**d** was always significantly higher than could be expected from the ratio [**1a**−**d**]:[CAN] in the starting reaction mixture (Table 1). The C5−C5′ linked dimer **4** might be formed in competition to the splitting reaction by elimination of a proton in **12**, followed by further oxidation and deprotonation of the radical intermediate **14**. The observation that in the reaction of $NO_3^{\bullet}$ with **1a** or **1d** the presence of excess oxygen or water, respectively, had no measurable influence on the product distribution and ratio could be taken as an indication for very fast reactions of the radical or radical ion intermediates. The apparent discrepancy in Table 1 that the dimers **1a** and **1b** are consumed equally fast in the reaction of the mixture of **1a**−**d** with $NO_3^{\bullet}$ (entry 1) and the comparatively low reactivity of **1b**, when it is reacted separately with $NO_3^{\bullet}$ (entry 3), can also be explained by the mechanism in Scheme 2. Since formation of the C5−C5′ linked dimer **4** is the major pathway in the reaction of **1b** with $NO_3^{\bullet}$, only minor amounts of the chain carrier **15** are produced, which could initiate the oxidation of further **1b**. Besides the shorter radical chain length the concentration of CAN is lower in the $NO_3^{\bullet}$ reaction with single **1b**: [**1b**]:[CAN] = 1.25 (entry 1) vs 5 (entry 3).

The $NO_3^{\bullet}$-induced splitting of the *anti* isomers **1c,d** is expected to proceed principally in the same way, although the products and their distribution are different. Their lower reactivity should be a result of their higher oxidation potentials (see Figure 1). In an independent experiment it was verified that the *N*-dealkylated uracils **8** and **9** result from a reaction of **2** with $NO_3^{\bullet}$. The dealkylation is expected to proceed through a stepwise electron transfer and deprotonation sequence [shown in Scheme 2 for the dealkylation at N(1)] and finally hydrolysis of the imminium species **17** by trace amounts of water, which are present in the system as a result of the hygroscopic CAN.[23] However, because of the present uncertainty in the structure assignment for compound **6**, we prefer not to speculate about the reaction pathway leading to its formation.

To conclude, we have demonstrated that the strongly oxidizing $NO_3^{\bullet}$ is able to cleave the cyclobutane dimers **1a**−**d** to the monomer **2** through an initial electron-transfer step. The rate of the splitting reaction and the splitting efficiency, as well as the distribution of the byproducts formed besides the monomer **2**, show a significant dependence on the stereochemistry and the substitution pattern at the cyclobutane ring. However, we do not know the reason for this behavior yet. Therefore, extensive studies on the reaction mechanism of the $NO_3^{\bullet}$-induced splitting of these and of further pyrimidine cyclobutane dimers are currently in progress in our laboratory.

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(20) Estimated value for the redox couple $NO_3^{\bullet}/NO_3^-$: $E^\circ = 2.0$ V vs SCE (in acetonitrile); see ref 10b.

(21) Whether or not the assumed $NO_3^{\bullet}$-induced electron transfer in **1** is either complete, leading to formation of a discrete radical cation **11**, or incomplete, where an intermediate charge transfer (CT) complex between the dimer and $NO_3^{\bullet}$ (not shown) may be suggested, is not clear. Pac et al. and Rosenthal et al. proposed (see refs 9 and 19) that a facile and selective splitting of the pyrimidine cyclobutane dimer may be achieved in a CT complex involving an intermediate with a partial positive charge developing on the pyrimidine dimer and a sensitizer, which acts as an electron acceptor. Therefore, the origin of the substrate selectivities should be due to steric effects. On the other hand, formation of the discrete radical cation of the dimer was found to lead to a rapid splitting independently of the structure at the cyclobutane ring.

(22) Pouwels, P. J. W.; Hartman, R. F.; Rose, S. D.; Kaptein, R. *Photochem. Photobiol.* **1995**, *61*, 563.

(23) The oxidative dealkylation of aromatic amines by $NO_3^{\bullet}$ has been studied by us: Krüger, O.; Wille, U., manuscript in preparation. An analogous dealkylation of amines by anodic oxidation was reported in the literature: Kyriacou, D. *Modern Electroorganic Chemistry*; Springer-Verlag: Berlin, 1994.