J|A|C|S
COMMUNICATIONS

Published on Web 02/19/2002

# Further Insight in the Photochemistry of DNA: Structure of a 2-Imidazolone (5−4) Pyrimidone Adduct Derived from the Mutagenic Pyrimidine (6−4) Pyrimidone Photolesion by UV Irradiation

Martial Thomas,[†] Dominique Guillaume,[§] Jean-Louis Fourrey,[†] and Pascale Clivio*,[†]

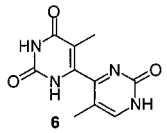
*Institut de Chimie des Substances Naturelles, CNRS, 1 Avenue de la Terrasse, 91 190 Gif sur Yvette, France, and Laboratoire de Chimie Thérapeutique, E.A. 2629, 1 Rue des Louvels, 80000 Amiens, France*

Received May 29, 2001; Revised Manuscript Received December 10, 2001

It has long been thought that DNA damage produced by UV light exerts much of its biological effect by interfering with DNA polymerase metabolism. This has led to numerous investigations of DNA-photolesion mutagenic properties currently intensified by the discovery of new DNA polymerases.[1] Recently, however, the picture has become more puzzling since new studies have highlighted the possible perturbation by DNA photodamage of other important DNA-dependent processes such as transcription, which may also have dramatic consequences for the cell.[2] The extreme complexity and the continuing discovery of new biological processes that are triggered or impaired by DNA photodamage consequently reinforce the need to further characterize the photochemistry of nucleic acids. Among the products of UV-induced DNA damage, (6−4) pyrimidine pyrimidone photoproducts that arise from a Paterno-Büchi reaction between two adjacent pyrimidine nucleobases are one of the major mutagenic class of DNA photoproducts[3] and are involved in the etiology of skin cancer.[4] Under UV A/B exposure, (6−4) photoproducts are converted into their also mutagenic[3a−b,d] Dewar valence isomers[5] which may play an important role in the biological effect of UV light.[6] Apart from Dewar adducts, no other secondary product derived from (6−4) photoproducts by a photochemical reaction has thus far ever been reported to occur in DNA or at the dinucleotide level. In light of the tremendous importance ascribed to (6−4) photoproducts and of the recent increasing interest devoted to the study of secondary nucleobase damage,[7] characterization of any secondary derived (6−4) photoproducts is of utmost importance and thus deserves particular attention.

Photoproduct analogues or their precursor have been essential to probe, in biologically relevant model reactions, the mechanistic formation or repair pathway of natural photoproducts.[8] In line with our initial effort in the PNA series,[9] we proposed to study the photochemical behavior of amide bond-containing dinucleotide mimics. Herein, we report the isolation and structural determination of a new photoproduct (**1**) isolated after 254 nm photolysis of the amide-linked thymidine dimer **2** (T*a*T),[10] an analogue of thymidylyl (3′-5′) thymidine (T*p*T). The structure of **1** shares the 3′-pyrimidone motif of the (6−4) adducts but, instead of the 5-hydroxy-6-substituted-dihydrothymine base portion, contains a 2-oxoimidazoline.

Thus, irradiation of an aqueous solution of **2** at 254 nm led, in addition to **1** (UV: $\lambda_{max}$ 338 nm, $\epsilon_{338}$ 3172 $cm^{-1}$ $mol^{-1}$), to the (6−4) adduct **3**, the Dewar photoproduct **4**, and the cyclobutane pyrimidine dimer **5** (Scheme 1).[11] Formation of compounds **3**−**5** demonstrated that **2** exhibits a photochemical behavior close to that observed for the natural T*p*T dimer[12] and consequently is a relevant model to study the photochemistry of nucleobases. The $^1$H NMR spectrum of **1** was partially reminiscent to that of (6−4) adduct **3** in displaying a characteristic pyrimidone H6 signal around 8 ppm[12] ($\delta$ 8.21 for **1**, 7.94 for **3**). However, the expected accompanying H6 signal of the saturated thymine residue was lacking, suggesting that **1** could be a 5,6-dehydrated (6−4) adduct whose base portion would correspond to the known Thy(6−4)m$^5$Pyo (**6**) accessible by



**6**

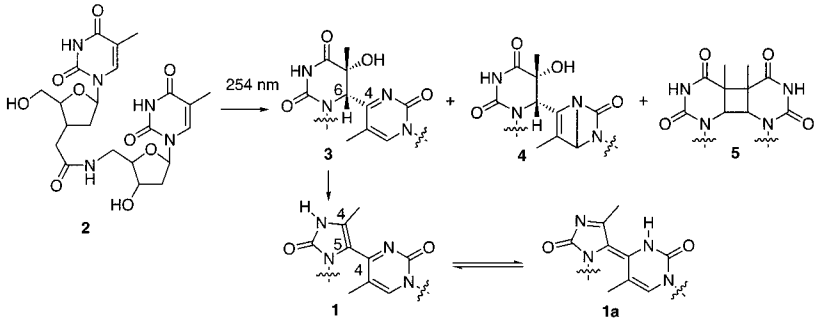
acidic hydrolysis of pyrimidine (6−4) pyrimidone adducts.[13] However, such hypothesis is unconsistent with the HR mass (FAB$^+$) data of **1** that revealed its molecular formula $C_{21}H_{28}N_5O_7$ (calcd 462.1989 ($MH^+$); found 462.1988). This demonstrated that the pathway leading from **2** to **1** included a dehydration reaction and the loss of a carbonyl group. The absence of this latter group was further confirmed by the $^{13}$C NMR spectrum of **1** that displayed 21 carbons including four carbonyl/imine resonances, instead of five in the case of **2** or **3**. Among these four carbons, the amide resonance was found at $\delta$ 176.3 ($^3J$ with *a*T H5′, H5″) indicating that the amide link between the sugars had been preserved during the photolysis step. Since the chemical shifts of the 11 remaining carbons belonging to the modified sugar phosphate backbone were also almost identical to the chemical shifts of the corresponding carbons of **2**, changes involved only the bases. Concerning these, all but one carbon of a 5-methylpyrimidone (C2, C5, C6, $CH_3$: $\delta$ 157.5, 118.6, 145.1, 15.1 respectively) could be rapidly identified since their chemical shifts closely matched those of **3** ($\delta$ 158.6, 118.4, 144.2, 15.5) and of the (6−4) adduct of T*p*T.[12,14] The only discrepancy concerned C4 ($\delta$ 168.0 for **1** vs 175.8 for **3**, and 175.6 for the (6−4) adduct of T*p*T)[12,14] that was unambiguously attributed from long-range (LR) heteronuclear NMR data. In addition, these data also permitted the localization of the 5-methylpyrimidone at the 3′-end of the molecule. Taken together, these observations led to the conclusion that the dehydration and decarbonylation concerned the 5′-end base and had led to the substitution of the sugar residue by a methyl-substituted cycle constituted of two nitrogen and three quaternary carbon atoms ($\delta$ 156.2, 123.2, 114.8), one of which was of the carbonyl/enamine type. The 2-oxoimidazoline nature of this cycle was deduced from the following key spectral NMR data. Carbon at 114.8 ppm gave a LR coupling with T*a* C$\underline{H}_3$ at $\delta$ 1.98 ($^3J$), T*a* H1′ at $\delta$ 5.97 ($^3J$) and *a*T H6 at $\delta$ 8.21 ($^4J$) and thus connected $N^1$ T*a* to C4 *a*T (C5 of the imidazolone). Such a substitution pattern was fully consistent with the 7 ppm shielding observed for the C4 atom of the 5-methylpyrimidone moiety.[15] The urea-type carbon at 156.2 ppm (C2 of the imidazolone) was positioned on $N^1$ T*a* since it gave a long-range coupling with H1′

* To whom correspondence should be addressed. Telephone: 33-1-69 82 30 63. Fax: 33-1-69 07 72 47. E-mail: pascale.clivio@icsn.cnrs-gif.fr.
† Institut de Chimie des Substances Naturelles.
§ Laboratoire de Chimie Thérapeutique.

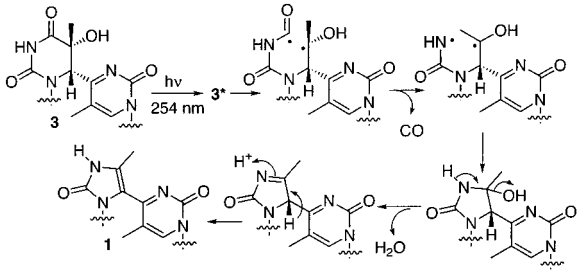


**10.1021/ja011292m CCC: $22.00** 

***Scheme 1***



***Scheme 2***



of the T*a* residue and was consequently linked to the remaining nitrogen ($N^3$ of the imidazolone). The carbon at $\delta$ 123.2 was hence C4 of the imidazolone, substituted, as attested by LR NMR correlation, by the remaining methyl group whose chemical shift (9.5 ppm) was in full accordance with the literature.[16] On the basis of these arguments, we assigned structure **1** to the new photoproduct.

Because of such a structure, we anticipated that **1** should be in tautomeric equilibrium with **1a**. Indeed, recording of the UV spectrum of **1** in alkaline conditions (pH12) led to a reversible ipsochromic shift ($\lambda_{max}$ 324 nm) and appearance of a second absorption ($\lambda_{max}$ 407 nm) that disappeared upon neutralization in agreement with the proposed structure.[17]

Due to structural similarities between **1** and **3**, we hypothesized that **3** could be the precursor of **1**. To gain more insight into the formation mechanism of **1**, we irradiated, at 254 nm, an aqueous solution of **3**. Examination of the $^1$H NMR spectrum of the crude irradiation mixture and HPLC analysis evidenced the formation of **1** and thus confirmed the lineage between **1** and **3**. Accordingly, the most likely pathway accounting for the formation of **1** might involve first the formation of **3**, then homolytic C4−C5 cleavage of the excited (6−4) adduct **3*** (Scheme 2). This step then gives rise to a biradical intermediate which undergoes spontaneous decarbonylation and then cyclizes to yield a five-membered ring carbinolamine intermediate whose aromatization leading to compound **1** is the driving force for dehydration.[16]

In conclusion, we have observed in the pyrimidine (6−4) pyrimidone photoproduct series a remarkable secondary photochemical pathway leading to a 2-oxoimidazoline (5−4) pyrimidone derivative. This constitutes the first report of a major photomodification affecting a (6−4) photoproduct and may put into question their biological stability in repair-deficient individuals submitted to prolonged UV-light exposure. Indeed, considering that (6−4) adducts are the second-most prevalent lesions formed in DNA, the probability that the 2-oxoimidazoline (5−4) pyrimidone derivatives may represent another source of endogenous DNA damage cannot be discarded and deserves further investigations.

**Supporting Information Available:** $^1$H, $^{13}$C, HMQC, HMBC NMR spectra of **1** as well as $^1$H NMR spectrum and $^{13}$C NMR data of **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

## References


(1) For reviews on recently discovered DNA polymerases, see: Goofman, M. F.; Tippin, B. *Mol. Cell. Biol.* **2000**, *1*, 101−109; Friedberg, E. C.; Feaver, W. J.; Gerlach, V. L. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5681−5683.

(2) Tommasi, S.; Swiderski, P. M.; Tu, Y.; Kaplan, B. E.; Pfeifer, G. P. *Biochemistry* **1996**, *35*, 15693−15703; Liu, X.; Conconi, A.; Smerdon, M. J. *Biochemistry* **1997**, *36*, 13710−13717; Vichi, P.; Coin, F.; Renaud, J.-P.; Vermeulen, W.; Hoeijmakers, J. H. J.; Moras, D.; Egly, J.-M. *EMBO J.* **1997**, *16*, 7444−7456.

(3) (a) LeClerc, J. E.; Borden, A.; Lawrence, C. W. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9685−9689. (b) Horsfall, M. J.; Lawrence, C. W. *J. Biol. Chem.* **1994**, *235*, 465−471. (c) Gentil, A.; Le Page, F.; Margot, A.; Lawrence, C. W.; Borden, A.; Sarasin, A. *Nucleic Acids Res.* **1996**, *24*, 1837−1840. (d) Smith, C. A.; Wang, M.; Jiang, N.; Che, L.; Zhao, X.; Taylor, J.-S. *Biochemistry* **1996**, *35*, 4146−4154. (e) Kamiya, H.; Iwai, S.; Kasai, H. *Nucleic Acids Res.* **1998**, *26*, 2611−2617.

(4) Sarasin, A. *Mutation Res.* **1999**, *428*, 5−10. Black, H. S.; deGruijl, F. R.; Forbes, P. D.; Cleaver, J. E.; Ananthaswamy, H. N.; de Fabo, E. C.; Ullrich, S. E.; Tyrrell, R. M. *J. Photochem. Photobiol., B* **1997**, *40*, 29−47.

(5) Taylor, J.-S.; Cohrs, M. P. *J. Am. Chem. Soc*. **1987**, *109*, 2834−2835.

(6) Perdiz, D.; Grof, P.; Mezzina, M.; Nikaido, O.; Moustacchi, E.; Sage, E. *J. Biol. Chem.* **2000**, *275*, 26732−26742.

(7) For selected examples, see: Duarte, V.; Gasparutto, D.; Yamaguchi, L. F.; Ravanat, J.-L.; Martinez, G. R.; Medeiros, M. H. G.; Mascio, P. D.; Cadet, J. *J. Am. Chem. Soc*. **2000**, *122*, 12622−12628 and references therein. Matray, T. J.; Haxton, K. J.; Greenberg, M. M. *Nucleic Acids Res.* **1995**, *23*, 4642−4648.

(8) Phosphorodithioate analogue: Murata, T.; Iwai, S.; Ohtsuka, E. *Nucleic Acids Res.* **1990**, *18*, 7279−7286; s$^5$(6−4) analogues: Clivio, P.; Fourrey, J.-L.; Gasche, J.; Favre, A. *J. Am. Chem. Soc.* **1991**, *113*, 5481−5483. Clivio, P.; Fourrey, J.-L. *Chem. Commun*. **1996**, 2203−2204. Liu, J.; Taylor, J.-S. *J. Am. Chem. Soc.* **1996**, *118*, 3287−3288; Formacetal analogues: Butenandt, J.; Eker, A. P. M.; Carell, T. *Chem. Eur. J*. **1998**, *4*, 642−654. Butenandt, J.; Burgdorf, L. T.; Carell, T. *Angew. Chem., Int. Ed. Engl*. **1999**, *38*, 708−711. Satou, K.; Komatsu, Y.; Torizawa, T.; Kato, K.; Shimada, I.; Nikaido, O.; Ohtsuka, O. *Tetrahedron* **2000**, *41*, 2175−2179. Schwögler, A.; Burgdorf, L. T.; Carell, T. *Angew. Chem., Int. Ed.* **2000**, *39*, 3918−3920; PNA analogue: Ramaiah, D.; Kan, Y.; Koch, T.; Ørum, H.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12902−12905.

(9) Clivio P.; Guillaume D. *Tetrahedron Lett.* **1998**, *39*, 6881−6884.

(10) De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Cook, P. D. Backbone Modified Oligonucleotide Analogues. International Patent SO 92/20,823, 1992. Idziak, I.; Just, G.; Damha, M. J.; Giannaris, P. A. *Tetrahedron Lett.* **1993**, *34*, 5417−5420.

(11) An argon purged 2 mM aqueous solution (100 mL) of **2** was irradiated for 100 h with a TNN 1532 Hanau lamp. Structure of **3**−**5** were established from UV, MS and NMR analysis (complete spectroscopic details will be published). Preparative purification was carried out by Reverse Phase HPLC using an Hypersil HS C18 column (5 $\mu$, 250 mm × 10 mm) and a 2% $CH_3CN$−water solution as eluent at a flow rate of 3 mL/mn. The isolated yields for **1**, **3**−**5** were 1; 19; 3.5 and 3%, respectively.

(12) Rycyna, R. E.; Alderfer, J. L. *Nucleic Acids Res*. **1985**, *13*, 5949−5963.

(13) Varghese, A. J.; Wang, S. Y. *Science* **1968**, *160*, 186−188. Douki, T.; Voituriez, L.; Cadet, J. *Chem. Res. Toxicol.* **1995**, *8*, 244−253.

(14) Quaternary carbons were assigned as follows: Carbon at $\delta$ 118.6 was attributed to *a*T C5 from its coupling with *a*T methyl at 2.14 ppm ($^2J$) and *a*T H6 at 8.21 ppm ($^3J$); *a*T C6 (144.2 ppm) from its coupling with *a*T H1′ at 6.28 ppm ($^3J$) and *a*T methyl at 2.14 ppm ($^3J$); *a*T C2 (158.6 ppm) from its coupling with *a*T H6 at 8.21 ppm ($^3J$); *a*T C4 (168 ppm) from its coupling with *a*T methyl at 2.14 ppm ($^3J$) and *a*T H6 at 8.21 ppm ($^3J$).

(15) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; Ebel, H. F., Ed.; VCH: Weinheim, 1987.

(16) Butler, A. R.; Hussain, I. *J. Chem. Soc., Perkin Trans II* **1981**, 310−316.

(17) The presence of an isobestic point (378 nm) supports a pH-dependent equilibrium between only two species.


JA011292M