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Photocycloaddition of 5-bromouracil to uracil in a dinucleotide model compound

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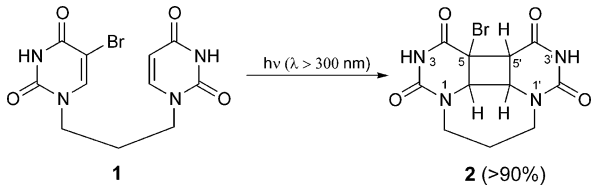
Abstract—The photochemical reactivity of 5-bromouracil towards uracil in a dinucleotide model compound **1** has been examined. The formation of a cyclobutane adduct **2** as a major product (>90%), without debromination of the 5-bromouracil moiety was observed upon irradiation of **1** with near UV light (λ >300 nm) under both anaerobic and aerobic conditions. Upon heating, **2** undergoes cyclobutane ring cleavage with concomitant hydrolysis to form 5-hydroxyuracil analogue **3** as a major product.

Photochemical reactions of 5-bromouracil (^{Br}U) have been studied extensively over the last 40 years. Most of these studies were directed towards understanding the mechanisms of various photochemical processes occurring in DNAs in which thymine residues had been replaced with ^{Br}U.[1,2] Among these processes, photoinduced strand breakage[3] and DNA–protein photocrosslinking[4] have attracted most attention, whereas photoreactions of ^{Br}U with nucleobases have been studied to a lesser extent. The major photoproduct from near-UV irradiation of DNA containing ^{Br}U was identified as 5,5′-diuridinyl.[5] The formation of other photoadducts with uracil, in addition to extensive debromination was observed in frozen aqueous solution or in dried films[6] as well as with thymine and cytosine in synthetic dinucleotides.[7] However, the structures of these photoadducts have not been fully assigned due to the difficulties encountered. The suggested formation of cyclobutane type adducts has been questioned,[8] and to the best of our knowledge no such adducts have been identified among the photoproducts isolated from irradiation of 5-bromouracil, until now. Previously, the formation of cyclobutane photoadducts of 5-fluorouracil[9] and 5-chlorouracil[10] with thymine and other pyrimidines was demonstrated using synthetic dinucleotides[9a] or dinucleotide model compounds in which the sugar–phosphate linkage was replaced by a trimethylene chain.[9b,10]

In the current study, the photochemical reactivity of a 5-bromouracil towards a uracil has been examined using similar dinucleotide model compound **1**[11] (Scheme 1).

An aqueous solution of **1** ($c=8\times10^{-4}$ M) was irradiated in a 0.2 cm UV cell using a 200 W high pressure mercury lamp equipped with a 313 nm interference filter under aerobic as well as anaerobic conditions. Both irradiations resulted in a gradual disappearance of **1**, with a slightly higher rate in the case of aerobic irradiation, as indicated by the observed decrease in the maximum at 272 nm (cf. Fig. 1). Under both conditions of irradiation, the formation of one major photoproduct was observed by HPLC up to 70% conversion of **1** and only small amounts of additional photoproducts could be detected in the solution irradiated to complete disappearance of **1** (Fig. 2). The characteristic UV spectrum of the major photoproduct exhibiting a lack of absorption maximum above 220 nm (Fig. 2, inset) as well as its photoreversion to **1** by irradiation at 254 nm, indicated a cyclobutane type photoadduct **2**.

To isolate and fully characterize the photoproduct by spectral methods, a 115 mg sample of **1** was dissolved



1 **2** (>90%)

Scheme 1.

Keywords: 5-bromouracil; photochemistry.

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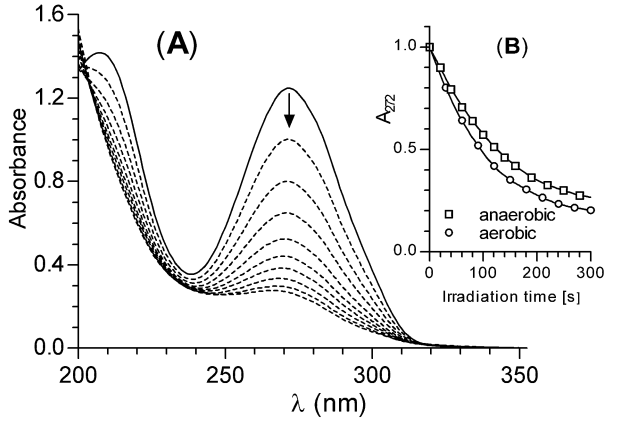

Figure 1. (A) Changes in the absorption spectrum of an aqueous solution of **1** during irradiation at 313 nm under aerobic conditions. The spectra were taken with 30 s time increments. (B) Comparison of the rates of disappearance of **1** under aerobic and anaerobic irradiations.

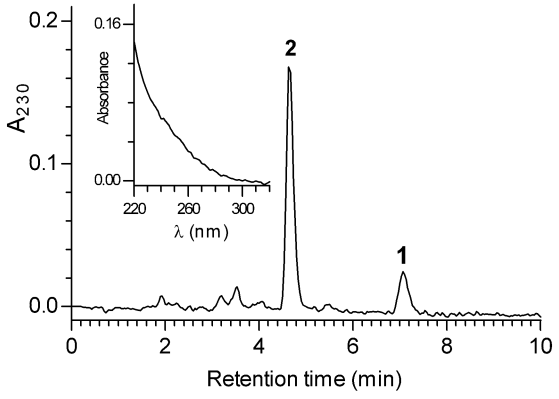

Figure 2. HPLC analysis of an aqueous solution of **1** after aerobic irradiation at $\lambda = 313$ nm to ca. 90% conversion. The column was a Waters XTerra RP_{18} 3.5 μm, 4.6×150 mm, eluted isocratically with acetonitrile/water (7:93, v:v) at a flow rate of 0.8 mL/min. The inset shows the UV absorption spectrum of the major photoproduct.

in 400 mL of water and the solution irradiated in portions in a 100 mL photoreactor with a 150 W immersed high pressure mercury lamp equipped with a Pyrex filter (λ >290 nm). The irradiations were continued until the complete disappearance of **1**. Combined irradiated solutions were concentrated under reduced pressure to ca. 10 mL and left overnight at 2°C. White precipitate was filtered off, washed with acetone and dried to give a chromatographically pure sample of **2** (74 mg, 64%). The ^{1}H and ^{13}C NMR and MS spectral data[12] are entirely consistent with **2** being an intramolecular cyclobutane photoadduct with the bromine substituent retained within the structure. Furthermore, close resemblance of the chemical shifts and splitting patterns of the cyclobutane ring H6 and H6′ protons as well as the methylene protons adjacent to the N1 and N1′ nitrogens with those of the previously obtained *cis–syn* cyclobutane photoadducts of other 1,1′-trimethylene-bridged pyrimidines,[9,10,13] indicates that **2** also has a *cis–syn* configuration. Photoirradiation of **2** at 254 nm results in its rapid photoreversion to **1**, reaching a pseudo photostationary state containing ca. 75% of the latter, as checked by HPLC. Continuation of the irradiation at this stage causes gradual conversion of **1** into other unidentified photoproducts. The structures of these photoproducts are currently being investigated.

The thermal stability of **2** has also been tested. It has been found that on refluxing in water, **2** undergoes cyclobutane ring cleavage as indicated by the appearance of an intense absorption band at 270 nm. A HPLC profile for the thermal degradation of **2** that reveal formation of one major product, **3**, in addition to some minor products is shown in Fig. 3.

The major decomposition product was isolated from the mixture by preparative HPLC and its structure was established based on the MS, NMR and UV spectral data.[14] The molecular formula of **3**, determined as $C_{11}H_{12}N_4O_5$ by HR MS, indicates that thermal cleavage of the cyclobutane photoadduct **2** leading to its formation occurs with concomitant hydrolysis of C5–Br. This is further supported by the appearance of an exchangeable singlet at δ 8.60 in the ^{1}H NMR spectrum of **3**, attributable to the OH proton of the 5-hydroxyuracil moiety.[15] Additional characterization of **3**, consistent with the proposed structure, was provided by the UV spectra in buffered aqueous solutions (not shown) which indicated a typical 5-hydroxyuracil chromophore[15] red shift in λ_{max} from ca. 272 to 303 nm on going from neutral to basic (pH 12) solution.

In summary, it has been demonstrated using a simple dinucleotide model compound, that 5-bromouracil undergoes 2+2 photocycloaddition with uracil upon irradiation with near UV light (λ >300 nm), without debromination to form thermally unstable cyclobutane adduct as a major photoproduct.

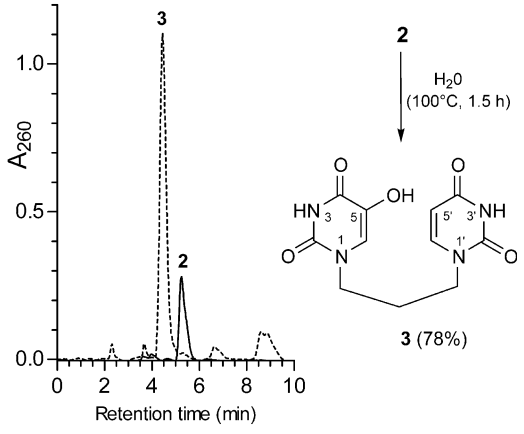

Figure 3. HPLC elution profile of a solution of **2** before heating (solid line) and after refluxing in water for 1.5 h (dotted line). The analysis conditions were analogous to those in Fig. 2 except for the flow rate, which was 0.7 mL/min. The inset shows the structure of the major decomposition product; HPLC-determined yield is given in the bracket.

Acknowledgements

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11. 5-Bromo-1-[3-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propyl]pyrimidine-2,4(1*H*,3*H*)-dione, **1**, was obtained as described previously. See: Dezor-Mazur, M.; Kazmierczak, F.; Golankiewicz, K. *Heterocycles* **1984**, *22*, 12.
12. 6b-Bromohexahydro-1*H*-3a,5,8,9a-tetraazacyclohepta-[1,2,3,4-*def*]biphenylene-4,6,7,9(5*H*,8*H*)-tetrone, **2**. Mp 292°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.90 (s, 1H, N3-H), 10.45 (s, 1H, N3′-H), 4.72 (d, 1H, C6-H, $J=6.3$ Hz), 4.48–4.45 (dd, 1H, C6′-H, $J=6.3$ Hz, $J=10.12$ Hz), 4.11–4.05 (m, 3H, N1-CH*H*, N1′-CH*H* and C6-H), 2.89 (m, 1H, N1-C*H*H), 2.80 (m, 1H, N1′-C*H*H), 1.80 (m, 1H, C-CH*H*-C), 1.56 (m, 1H, C-C*H*H-C). ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.65, 163.44, 150.48, 150.27, 64.44, 52.79, 48.05, 47.74, 46.60, 45.95, 24.06. FAB MS m/z 343.00 and 345.00 $(C_{11}H_{11}N_4O_4{}^{79}Br+H)^+$ and $(C_{11}H_{11}N_4O_4{}^{81}Br+H)^+$. Anal. calcd for $C_{11}H_{11}BrN_4O_4$: C, 38.50; H, 3.23; N, 16.33. Found: C, 38.39; H, 3.16; N, 16.28%.
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14. 1-[3-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propyl]-5-hydroxypyrimidine-2,4(1*H*,3*H*)-dione, **3**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.38 (s, 1H, N3-H), 11.23 (s, 1H, N3′-H), 8.60 (s, 1H, C5-OH), 7.65 (d, 1H, C6′-H, $J=7.7$ Hz), 7.19 (s, 1H, C6-H), 5.53 (d, 1H, C5′-H, $J=7.7$ Hz), 3.63 (m, 4H, N1-CH_2 and N1′-CH_2), 1.87 (m, 2H, C-CH_2-C). ^{13}C NMR (HETCOR) (DMSO-d_6): δ 163.54, 160.74 (C4, 4′), 150.77, 149.23 (C2, 2′), 145.37 (C6′), 132.09 (C5), 124.51 (C6), 100.83 (C5′), 45.01, 44.58 (N-*C*-C-*C*-N), 27.98 (N-C-*C*-C-N). HR MS calcd for $C_{11}H_{12}N_4O_5$: 280.0808. Found: M^+ 280.0810. UV (H_2O, pH 7.0) λ_{max} 272 nm, (pH 12.0) λ_{max} 262 and 303 nm.
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