The Photoreactions of Thymine with Hypoxanthine and Imidazole*

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Photocycloaddition, Thymine, Azetidine, Cyclobutane Adducts

Photochemical reactions of thymine linked to hypoxanthine or imidazole by a trimethylene chain were studied in aqueous solution. Irradiation (λ = 254 nm) of thymine-hypoxanthine pair yielded two internal cycloadducts with azetidine and cyclobutane part structures. Sensitization and quenching experiments suggested that the excited singlet was the reactive state in the photocycloaddition reactions. Only cyclobutanes were isolated from irradiated (λ = 290 nm) solutions of thymine-imidazole pairs. Photocycloadditions were reversible upon irradiation at λ = 254 nm.

Introduction

(2+2) Photocycloaddition of thymine, a pyrimidine base of nucleic acids is well documented. Thymine photodimerization has been subjected to extensive studies as the dimers account for most of photobiological effects observed upon UV irradiation of cells [1, 2]. Dimers are not the only cyclobutane-type products of thymine. Simple alkenes, vinyl esters and ethers [1], alkynes [3], acetylenic esters [4], coumarins and psoralens [5] when undergo photoaddition to pyrimidines give cyclobutanes as well.

For several years we have been studied the reactions of thymine irradiated in the presence of some compounds of biological importance. Bichromophoric systems in which thymine was linked to a respective compound by a trimethylene chain were used in these studies. Previously, we have described the photocycloaddition of thymine and adenine leading to a novel adduct with azetidine part structure [6, 7].

The investigation of the photoreactions of thymine-hypoxanthine pair was carried out as a continuation of the previous studies. Hypoxanthine is a minor base found in certain ribonucleic acids [8]. In this paper the photoreactions of thymine-imidazole pairs are also described. These reactions may be relevant to the interaction of proteins and nucleic acids under irradiation. The naturally occurring aminoacid-histidine (α-amino-lH imidazole-4-propanoic acid) was shown to react with uracil under UV irradiation [9]. There is, however, no decisive information about the structure of the photoproduct(s).

Results and Discussion

Thymine and hypoxanthine

Aqueous solutions of 1a, b were irradiated with low-pressure mercury lamp (λ = 254 nm) until no further changes in the absorption spectrum were observed (10 min, Fig. 1).

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Fig. 1. UV spectra of 1a, (——) before (1), (— — —) after irradiation at λ = 254 nm for 3 min (2), 6 min (3), 9 min, 30 min (4).

Solvent evaporation (at ~ 50 °C) followed by column chromatography (ambient temperature) gave photoproducts 2 and respective substrates 1 in the ratio of ca. 1:9 (Scheme 1). Under those conditions cyclobutanes 2 were the only stable photoproducts [10].

However, the UV spectrum of 2 (Fig. 2) indicated that the direct formation of this product under UV irradiation could not account for the absorbance changes presented in Fig. 1. Thus, some other unstable products must also be present in the reaction mixture.
Photoreactions of 1a were studied in detail. Two major photoproducts: 3 (R f 0.21, solvent system A, Experimental) and 4 (R f 0.29, A) in addition to the substrate 1a (R f 0.36, A) were detected by TLC immediately after irradiation. Some traces of 2a (R f 0.56, A) were observed only.

Azetidine 4 (Scheme 1) was isolated from the reaction mixture when the temperature was carefully controlled and kept below 10 °C throughout. Photoproduct 3 was found to undergo a dark reaction during workup to yield 2a. Therefore 3 was obtained only in a small quantity by a column chromatography on silica gel. The tentative assignment of the structure of 3 (Scheme 1) followed from the chemical and photochemical properties of the photoproduct.

The compound 2a was shown (TLC, UV) to be formed in the dark after 3 had been treated with water (Fig. 3). Nucleophilic addition of a water molecule in the dark to the C=N bond and ring opening are well-established reactions for 5,6-dihydro-4-oxopyrimidines [11]. Moreover, the product 3 like azetidine 4, converted back to the substrate 1a after reirradiation at \( \lambda = 254 \) nm (Fig. 3).

![Fig. 2. UV spectra of 1a ( ), 2a ( •••••• ) and 4 ( ) in H2O.](image)

![Scheme 1](image)

The quantum yield for 1a disappearance was estimated to be 0.05 (\( \lambda = 254 \) nm) and is of the same magnitude for 1b. Similar scheme presumably describes the photoreactions of 1b. The composition of the photoequilibrium mixture 1a:3:4 was estimated from the UV spectrum to be 7:1:2. The concentration of the product 3 was calculated using molar extinction coefficients determined in H2O–MeOH solution (\( \lambda_{\text{max}} \) 227 nm, \( \varepsilon_{\text{max}} \) 11,000, Fig. 3). The photoproduct 2a was obtained with a higher yield (25%) under irradiation of 1a with \( \lambda > 265 \) nm.

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order as that found for intramolecular photodimerization of trimethylenebisthymine [12]. The photocleavage reactions: 4→1a (Φ = 0.43 at 254 nm, Φ = 0.47 at λ = 313 nm) and 3→1a (Φ ~ 0.3 at λ = 254 nm, determined in MeOH) proceeded with much higher efficiencies. Acetone could not sensitize the formation of the products 4 and 2a. The triplet energy of acetone is higher than that of nucleotide bases and it has been reported previously that triplet acetone was quenched by oxopurines and thymine mainly via triplet-triplet energy transfer [13]. Additionally, oxygen, the well known triplet quencher [14] was found not to affect the rate of disappearance of 1a. Thus, both sensitization and quenching experiments suggested that the excited singlet was the reactive state in photocycloaddition reactions of 1a.

The structures of the isolated photoproducts 2a, 2b and 4 were established on the basis of spectral data. The rigid, cyclobutane-type structure of the products 2a and 2b was evident from the NMR spectra. The aromatic atom resonances typical for purine and pyrimidine rings [15] were not observed in the 13C NMR spectrum of 2a. The 1H NMR spectrum of 2a is presented in Fig. 4. The signals were assigned to respective protons by double resonance, exchange with D2O and a comparison of the chemical shifts to those of compounds alike.

The spectrum indicates that formamido group of 2a exists in CDCl3 solution predominantly in the syn form (J NH,NCH3 = 1.5 Hz). This form has been shown to be favoured for N-monosubstituted aliphatic amides including N-t-butylformamide [16]. Both rotational isomers are present in DMSO-D2O solution because two formyl (Hβ) signals at δ 8.18 and δ 8.23 have been observed. The dependence of the isomer ratio on the solvent has been observed previously [17]. The 1H NMR spectrum of 2a also indicates that in CDCl3 solution NH proton of the formamido group is involved in intramolecular hydrogen bonding most likely with C=O of the ortho substituent. This proton gives rise to the concentration (0.90–0.025 M) independent signal shifted about 2 ppm downfield as compared with the NH proton signal of N-t-butyl aliphatic amides in nonpolar solvent [16]. In more polar solvent-DMSO these intramolecular bonds are broken and intermolecular hydrogen bonds with solvent molecules are formed. The NH signal appears at δ 9.16 (0.15 M, DMSO-d6) and its chemical shift is concentration dependent.

In the electron impact mass spectrum of 2a and 2b the major fragment is at m/z 320 and 292, respectively, indicating the loss of CO from molecular ion to be the major fragmentation pattern. The peaks corresponding to molecular ions are the major peaks in the field-desorption mass spectra of 2a and 2b.

The structure of the product 2a has been further confirmed by thermal and photochemical transformations. Heating 2a at 210 °C for 3 h gives the sub-

![Fig. 4. 1H NMR spectrum of 2a in CDCl3.](image-url)
strate 1a. Irradiation with light of wavelength $\lambda = 254$ nm cleaves a cyclobutane ring (eq. (2)).

$$\begin{align*}
2a & \xrightarrow{\text{hv}} 1a \\
\text{hv} & \text{(254 nm)} \\
5
\end{align*}$$

(2)

The resulting product 5 (Scheme 2) was identified by a comparison (TLC, NMR, UV) with an independently synthesized sample. Similarly, the cleavage of a cyclobutane ring of 2b occurred since the irradiation resulted in increasing of absorbance at $\lambda \approx 250$ nm. The quantum yield for the appearance of 5 was estimated to be 0.50 at $\lambda = 254$ nm. It should be pointed out that the initially formed compound 5 undergoes further photochemical reaction under prolonged irradiation (see below).

The remaining photoproduct, azetidine 4, is thermally unstable. It converts to the starting 1a by heating at 50 °C with $t_1/2$ of 100 min. The photoproduct 4 is also unstable in acidic and basic solutions. These properties of the product 4 are identical with those of the previously described azetidine derivatives [6, 7]. The $^1$H NMR spectrum of 4 confirmed the suggested structure. Cycloaduct 4 is 4,5-diaminopyrimidine derivative and therefore it shows, in the UV spectrum, the long wavelengths absorption band (Fig. 2) [18].

Unfortunately, the spectral data are insufficient for the definite assignment of the stereochmistry of the cycloadducts. Careful inspection of Dreiding models suggests cis-syn geometry for 2 and 4 due to the shortness of the trimethylene chain [19].

Photocycloadditions of carbon-nitrogen double bonds are relatively rare [20]. Photoreactions involving the “inner” C=C bond of oxopurines have been reported recently [21, 22].

**Thymine and imidazole**

In the case of thymine-imidazole pairs one would expect the competitive addition of thymine C=C bond to C=C and C=N bonds of imidazole moiety. However, irradiation of 5 and 6 through Pyrex ($\lambda > 290$ nm) gave only cyclobutanes 2a and 7, respectively (Scheme 2).

The photocycloaddition was a photoreversible process; substrates were obtained upon irradiation of cyclobutanes 2a and 7 at $\lambda = 254$ nm. In the case of the compound 5 a slower, irreversible photodecarbonylation leading to 8 competed with photocycloaddition (Scheme 2). The product distribution (2a and 8) was dependent on irradiation wavelength. Cyclobutane 2a was the major product (90% yield based on 5 reacted) accompanied by the traces of 8 when 5 was irradiated with $\lambda > 290$ nm. Photoproduct 8 could be obtained in quantity by irradiation of 5 at $\lambda = 254$ nm. In the latter case, initially formed cyclobutane 2a was converted back to 5, and since 8 was stable to irradiation, it was the eventual product (see Experimental).

The structures of all the photoproducts were confirmed spectroscopically. The NMR coupling constants (see Experimental) and inspection of Dreiding models suggest that the cycloadduct 7 is cis-syn isomer [19, 23, 24].

The photocycloaddition of imidazole derivatives has been reported previously. The imidazole C=C bond has been suggested to be involved in the formation of oxetanes in Paterno Büchi reaction of aliphatic and aromatic ketones [25] and of dioxetanes in dye sensitized photooxidation [26]. From the study described here it seems likely that photocycloaddition may contribute to a covalent linking of histidine and pyrimidine bases.

**Experimental**

Melting points were uncorrected. Ultraviolet spectra were recorded on a Zeiss UV-VIS spectrophotometer. Twice distilled water was used as a solvent. IR spectra were obtained on a Perkin
Elmer 580 spectrophotometer by using KBr pellets. 1H NMR, 13C NMR spectra were measured on Varian A 60 and Jeol FX 90 Q spectrometers with TMS as the internal standard. Electron impact mass spectra (MS) were determined at 70 eV with Jeol JMS-D-100 and field-desorption mass spectra (FD-MS) with Varian MAT 311 A spectrometers. Microanalyses were made on Carlo Erba or Perkin 240 Elemental Analysers. Serva silica gel 200—300 mesh was used for preparative thick layer chromatography (2 mm thickness) and for TLC analysis (0.20 mm thickness) Solvent systems: A. EtOAc—MeOH (1:1), B. EtOAc—MeOH (5:1), C. CHCl3—MeOH—HCOOH (7:2:1), D. CHCl3—MeOH (5:1), E. CHCl3—MeOH (30:1), F. CHCl3—MeOH (3:1),

9-[3-(Thym-1-yl)propyl] hypoxanthine 1b was synthesized as described previously [27]. The compound 1a was prepared from 1b by methylation (CH3I, NaH, DMF). Mild alkaline hydrolysis of 1a (0.125 M NaOH in H2O—EtOH, room temp., 4 h) gave 5. The compound 6 was synthesized by alkylation of imidazole with l-(3-bromopropyl)thymine. All new compounds 1a, 2a, 5, 6 exhibited consistent spectra (NMR, UV, IR, MS) and elemental analyses.

Preparative irradiations

The preparative irradiations were carried out in a cylindrical reactor using Original Hanau immersion mercury lamps: low-pressure TNN 15/32 (A = 254 nm) and high-pressure TQ 150 provided with a cylindrical Pyrex filter (A >290 nm). A solution filter (0.5 cm layer) of potassium iodide in water (1%, w:v) and high-pressure lamp was used for A >265 nm irradiations. Irradiations were performed on 0.50—0.30 g scale using 1 mM aqueous solutions. All chemical yields reported are the yields of isolated compounds.

Irradiation of 1a: The photolyzed solution of 1a (A = 254 nm) was concentrated in vacuo. The precipitate of unreacted 1a was filtered off and the filtrate was recovered in 85% yield. 2a was isolated with 25% yield from A >265 nm irradiated 1a; m.p. 220—223 °C.

IR cm⁻¹: 3420, 3240 (NH), 1700, 1670 (C=O).

MS m/e (rel. int.): 348(2), 320(100), 289(8), 181(57), 167(5), 164(9), 154(47), 153(9), 140(9).

FD-MS: 348(100).

13C NMR (CDCl3) δ: 170.01, 168.93, 161.39, 159.56, 151.21 (C=O and C=N), 79.10, 78.56, 75.74, 63.66, 48.11, 46.54, 43.39, 28.66, 27.57, 25.68, 21.50.

UV (Fig. 2). 1H NMR (Fig. 4).

Analysis for C15H18N4O4
Calcd C 51.70 H 5.79 N 24.13,
Found C 51.72 H 5.76 N 24.23.

4: In a separate experiment azetidine 4 was isomerized from the CHCl3 extract by preparative thick layer chromatography (A). The plates were developed twice. Temperature was kept below 10 °C during the workup.

1H NMR (DMSO-d6) δ: 7.80 (s, 1, HC=N), 5.77 and 4.27 (2 d, J = 5 Hz, 2, azacyclobutane H), 4.10—3.50 and 2.30 and 1.50 (m, 6, CH2CH2CH2), 3.40 and 2.96 (2 s, 6, N—CH3), 1.71 (s, 3, CCH3).

UV (Fig. 2).

Analysis for C13H16N6O4
Calcd C 51.74 H 5.94 N 25.45,
Found C 51.72 H 5.62 N 25.12.

Irradiation of 1b: The irradiated (A = 254 nm) solution was concentrated. The precipitate of unreacted 1b was filtered off and the filtrate applied on a silica gel plate. After drying under vacuum silica gel was placed on the top of a chromatographic column. Elution (D) gave 2b which crystallized from the concentrated eluate (10% yield). Starting material 1b was recovered in 80% yield.

2b: m.p. 305—306 °C (dec).

UV λmax 223 nm (ε 5, 340), (0.1 N KOH) λmax
239 nm (ε 8, 170).

IR cm⁻¹: 3400, 3280, 3190 (NH), 1720, 1700, 1670 (C=O).

1H NMR (DMSO-d6) δ: 10.32 and 9.21 (2 s, 2, N—H), 8.25 and 8.21 (overlapping d, total 1, CHO), 7.36 (s, 1, HC=N), 6.48 (br, s, 2, CONH2), 4.14—2.08 (m, 7, cyclobutyl H, CH2CH2CH2 overlapping with solvent), 1.49 (s, 3, C(CH3)2).

MS m/e (rel. int.): 292(100), 275(27), 154(6), 150(27), 140(51), 136(16), 126(16), 123(68), 109(16).

FD-MS m/e (rel. int.): 320(100).

Analysis for C15H18N6O4
Calcd C 48.73 H 5.04 N 26.24,
Found C 48.41 H 4.96 N 26.02.

Irradiation of 5: The irradiated (Pyrex) solution of 5 was concentrated and extracted with CHCl3. Column chromatography (B) of the CHCl3 extract gave the photoproduct 2a (47% yield) followed by the
starting material 5 (48% yield). Traces of 8 were detected.

When 5 (0.5 mmol) was irradiated at \( \lambda = 254 \text{ nm} \) the reaction was followed by TLC (C) and carried to ca. 50% conversion of 5. The isolation procedure as described above. 2a (ca. 3% yield) was eluted first from the column, then the major photoproduct 8 (52% yield) was obtained, followed by the starting material 5 (42% yield). Prolonged irradiation gave 8 with 73% yield and 5 (20% yield).

Quantum yields, sensitization and quenching experiments

Irradiations were carried out on an optical bench using low-pressure mercury lamp (\( \lambda = 254 \text{ nm} \)) or high pressure mercury lamp HBO 200 and a combination of interference (Zeiss) and glass BC-4 (Mash-priborintog, USSR) filters (\( \lambda = 313 \text{ nm} \)). Uranyl oxalate was used as an actinometer [28]. Aqueous solutions of 1a (0.2 mM) 4 and 2a (1 mM) were deoxygenated and irradiated in a cuvette. In the case of 3 a solution in MeOH (0.3 mM) was used. In quenching experiment oxygen was bubbled through the solution of 1a. The amount of the material that underwent the reaction was determined from UV measurements. The irradiations were carried out to a low conversion of the starting materials (<10%) and the calculated quantum yields were extrapolated to zero irradiation time.

In the sensitization experiment the solution of 1a (1 mM) in water-acetone mixture (1:1) was deoxygenated and irradiated at \( \lambda = 313 \text{ nm} \). Under these conditions acetone absorbs 99% of the incident light. The solvent was evaporated and a residue subjected to TLC analysis.

Thermolysis of 2a

A sample of 2a in a NMR tube was heated at 210 °C (oil bath) for 3 h. Then, DMSO-d_6 was added and \(^1\)H NMR spectrum recorded. NMR spectrum and TLC of the reaction mixture indicated the presence of 1a.

Photocleavage of the cycloadducts

Solutions (1 mM) of 2a, 4 and 7 were deoxygenated and irradiated in the reactor at \( \lambda = 254 \text{ nm} \) (2a, 4, 7) or through Pyrex (4). The reactions were followed by UV spectroscopy. Water was evaporated off and the reaction mixtures were analyzed by TLC and \(^1\)H NMR spectroscopy.
[19] The nomenclature used here is that generally used for pyrimidine dimers, see ref. [2], p. 227.
[23] The relative signs of the coupling constants of cyclobutane ring protons (see ref. [24]) could not be determined in spin tickling experiment because of the partial overlapping of cyclobutane and trimethylene bridge proton signals.