



pH DEPENDENT PHOTOOXYGENATION OF GUANINE BY SINGLET OXYGEN IN PRESENCE OF ROSE BENGAL

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ABSTRACT

Photooxygenation of guanine has been studied in presence of rose bengal. The effect of variation of pH has been studied on the product yield and time of the reaction. The reaction has been monitored by TLC at different time intervals. Backstrom type II mechanism has been proposed for these reactions.

Keywords: Photooxygenation, Guanine, Singlet oxygen, pH.

INTRODUCTION

Photooxygenation of organic compounds by singlet oxygen has been extensively studied in recent years¹⁻¹⁵. The photooxygenation of heterocycles leads to a variety of products and serves as an important tool in the synthesis of many natural products or compounds of special interest. Photooxidative guanine nucleobase is easily damaged amongst the four DNA bases. It is most easily oxidisable base. This is explained by the fact that guanine has the lowest ionization potential.¹⁶ Although many studies have been made and are being made to isolate and characterize intermediates and products, the mechanism of photosensitized oxidation of biomolecules as histidine or DNA bases is still not completely clear. A great deal of literature survey reveals that quite a lot work has been done on the photochemistry of guanine bases under different conditions,¹⁷⁻²¹ but a negligible attention has been paid to photooxygenation of guanine by singlet oxygen in presence of rose bengal at different pH. Therefore, in the present article, we report the pH dependent photooxygenation of guanine by singlet oxygen in presence of rose bengal.

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EXPERIMENTAL

Photooxygenation of guanine by singlet oxygen in acidic medium

To the solution of guanine (2.0 g.) in distilled water (100 mL) and methanol (100 mL), rose bengal (0.01 g.) was added as sensitizer. The solution was made acidic (pH 5.8 and 2.4) by adding dil. HCl. Then the solution was irradiated with low-pressure mercury vapor lamp, placed inside the immersion well photoreactor. Air was continuously bubbled through the solution with the help of an aerator. The progress of the reaction was monitored by TLC using benzene : methanol (2 : 3) solvent system. The irradiation was stopped after completion of the reaction as indicated by TLC. Then the dye was removed by animal charcoal treatment. The solution was concentrated on water bath under reduced pressure and then the beaker was placed as such overnight in refrigerator. The solid appeared, was filtered, washed with water and recrystallised with ethanol to give colorless crystals (**I**).

Found: C 36.39%, H 3.01%, and N 28.32%; Calculated for $C_6H_6N_4O_4$: C 36.37%, H 3.05%, and N 28.28%.

Photooxygenation of guanine by singlet oxygen in neutral medium

To the solution of guanine (2.0 g.) in distilled water (100 mL), methanol (100 mL), rose bengal (0.01 g.) was added as sensitizer. Then the neutral solution was irradiated with low-pressure mercury vapor lamp, in the immersion well photoreactor. No change was observed on TLC even after 50 hrs of irradiation. Therefore, the reaction was stopped.

Photooxygenation of guanine by singlet oxygen in alkaline medium

To the solution of guanine (2.0 g.) in methanol (100 mL), rose bengal (0.01 g.) was added as sensitizer. The solution was made alkaline (pH 8.5 and 9.8) by adding dil. NaOH. The same procedure was adopted as in acidic medium. The solid appeared on completion was filtered, washed with water and recrystallised with ethanol to give colorless crystals (**II**).

Found: C 36.48%, H 3.56%, N 35.51% Calculated for $C_6H_7N_5O_3$: C 36.55%, H 3.58%, and N 35.52%.

RESULTS AND DISCUSSION

Photooxygenation of guanine in acidic medium

When a methanolic acidic solution of guanine is irradiated by UV light in presence

of singlet oxygen at pH 5.8 and 2.4, it undergoes protonation and simultaneously, it forms peroxide by the addition of singlet oxygen. The peroxide linkage then dissociates and combines with methanol along with deamination to give the product **(I)**. The identity of the product has been established by elemental and spectral analysis. IR spectrum of the product **(I)** shows the following important peaks-3417 cm^{-1} (N-H stretch.), 1706 cm^{-1} (C=O stretch.), 1660 cm^{-1} (C=N stretch.), 1328 cm^{-1} (C-O), 1199 cm^{-1} (C-N stretch) etc. The ^1H NMR spectrum of product **(I)** shows peaks at δ 10.80 (-NH proton, five membered ring), 8.05 (-NH proton, six membered ring), 5.43 (-OH proton), and 3.91 (-OCH₃ protons). The ^{13}C NMR spectrum of the product **(I)** shows the peaks at δ 164(C₁), δ 166(C₂), δ 174(C₃), δ 160(C₄), and δ 154(C₅), and δ 55 (C₆) (Fig. 1).

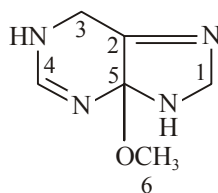


Fig. 1

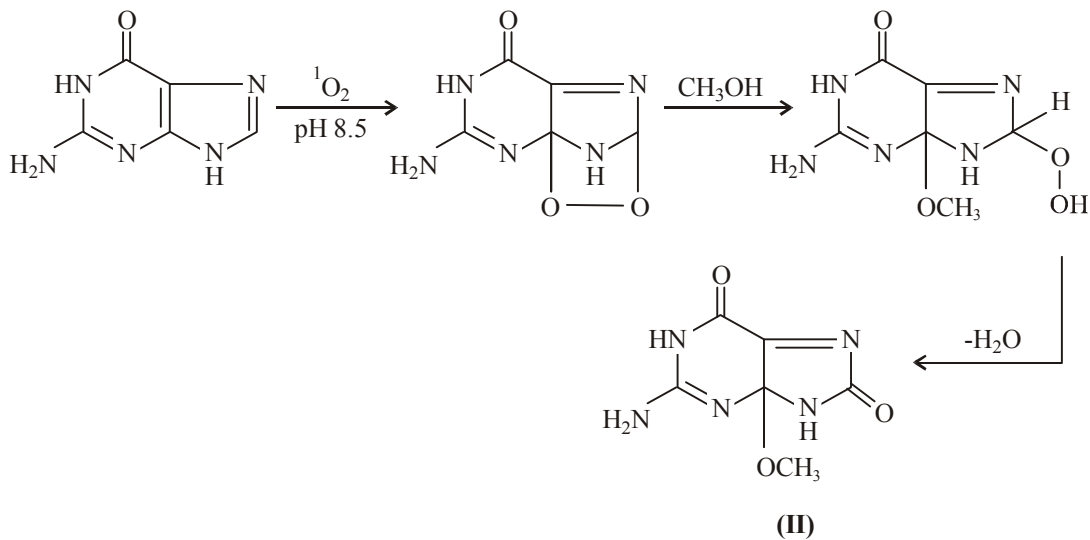
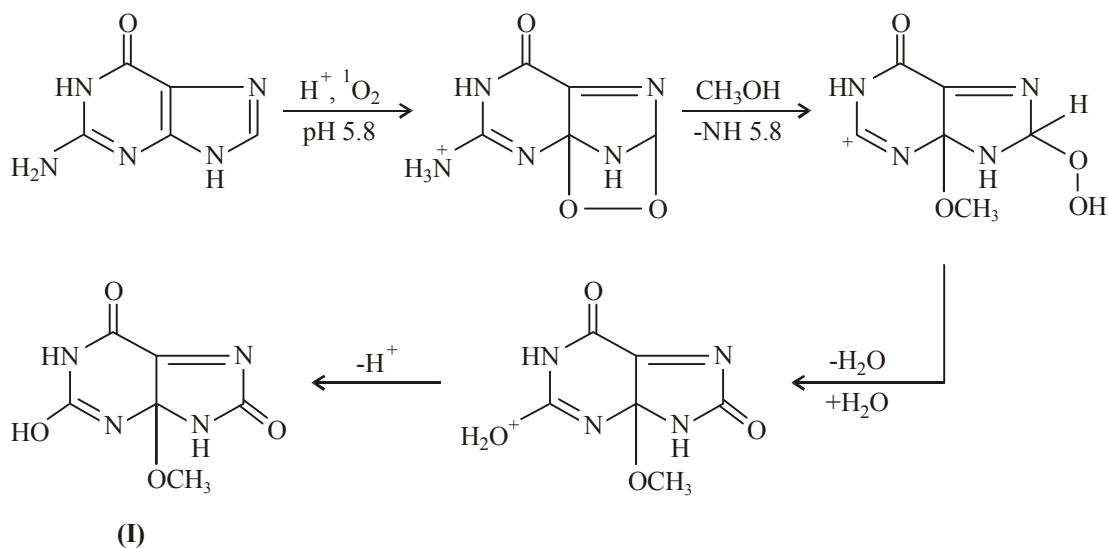
The mass spectrum of the product **(I)** shows molecular ion peak at m/e 198 and the other important peaks at 156, 152, 150, 138, 109, 81, 54 etc.

Photooxygenation of guanine in alkaline medium

When an alkaline solution of guanine in methanol was irradiated by UV light in presence of singlet oxygen at pH 8.5 and 9.8, it gives product **(II)** through the formation of endoperoxide as shown in **Scheme 2**. The structure of the product **(II)** has been confirmed by spectral data and elemental analysis. IR spectrum of the product **(II)** shows the following important peaks at 3342 cm^{-1} (N-H stretch.), 2920 cm^{-1} (C-H stretch), 1693 cm^{-1} (C=O stretch), 1654 cm^{-1} (C=N stretch), 1234 cm^{-1} (C-O stretch) etc. The ^1H NMR spectrum of product **(II)** shows peaks at δ 11.50 (NH proton, five membered ring), 8.0 (NH proton, six membered ring), 6.54 (-NH₂ protons), and 3.86 (OCH₃ protons). The ^{13}C NMR spectrum of the product **(II)** shows the peaks at δ 164(C₁), δ 166(C₂), δ 174(C₃), δ 160(C₄), and δ 154(C₅), δ 52 (C₆). (Fig. 1) The mass spectrum of the product **(II)** shows molecular ion peak at m/e 197 and other important peaks at m/e 166, 150, 106, 80 etc.

The participation of singlet oxygen in the reaction has been confirmed by carrying out the reaction in presence of β - carotene as singlet oxygen scavenger, whereupon no reaction takes place. The reaction has been carried out at two different pH in acidic and alkaline ranges and it has been observed that not only, different products were obtained at

different pH in acidic range and in alkaline range but the yield of the product and the time taken for completion of reaction were also different (Table 1).



CONCLUSION

Guanine is found to be inactive or stable in neutral condition, but can be oxidized photochemically in acidic and alkaline media by singlet molecular oxygen. Different

products were obtained in acidic and alkaline media. The reaction is fast at pH 5.8 and 9.8 compared to pH 2.4 and 8.5, respectively; thus, indicating that on increasing the pH, the rate of the reaction also increases.

Table 1

pH	Time (hrs)	Product	Melting point (°C)	Yields (g)
5.8	34	(I)	300-302	~1.9
2.4	38	(I)	300-302	~1.6
Neutral	50	No reaction	-	-
8.5	43	(II)	296	~1.1
9.8	40	(II)	296	~1.5

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