PHOTOREDUCTION OF PYRIMIDINE NUCLEOSIDES IN THE PRESENCE OF SULFITE ION

Yoshikazu Kondo*, Fumihiko Yoshizaki, and Tsunematsu Takemoto

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, Japan

Irradiation of aqueous solutions of uridine and thymidine at pH 9 with 2537 Å-light in the presence of bisulfite revealed complete disappearance of the uv absorption. Hydrolysis followed by purification on silica gel column chromatography gave the corresponding dihydropyrimidine bases. In this reaction of thymidine 2-oxo-4-methoxy-5-methylhexahydropyrimidine was obtained in addition to dihydrothymine. Excess bisulfite was unaffected to the resulting dihydropyrimidine nucleus.

In the photoreduction of uridine and thymidine to the corresponding dihydroderivatives, it was found that sulfite ion was sufficiently mild photochemical reductant when the irradiation was carried out in aqueous solutions of sodium[1] or ammonium bisulfite buffered at pH 9. Under neutral or acidic conditions, however, no photoreduction was observed.

In a typical spectroscopic run a 10^{-3} *M* buffered solution (pH 9) of pyrimidine nucleoside was irradiated at ambient temperature with a Hanovia low-pressure mercury lamp (No. 87A-45, intensity of 4.3 W at 2537 Å) in the presence of ten molar equivalents of sodium or ammonium bisulfite. Aliquots were withdrawn at intervals and the optical density was measured at the respective absorption

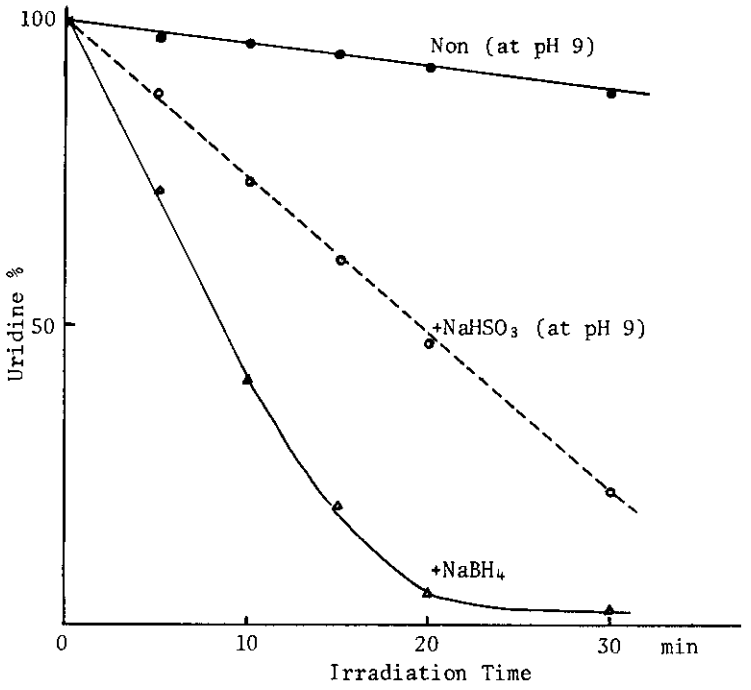

Fig. 1. Photoreduction of Uridine.

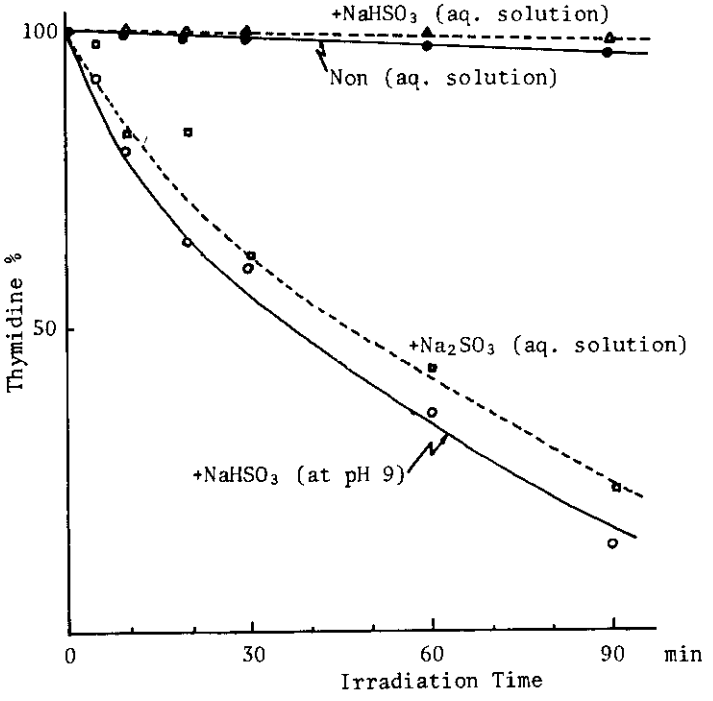

Fig. 2. Photoreduction of Thymidine.

maximum. As shown in Fig. 1, uridine revealed practically no photohydration at pH 9. In fact, the photoreaction was irreversible because of the absorption could be unrecovered by heating of the reaction mixture at 100° for 1 hr. Likewise, thymidine lost its uv absorption to the desired extent when the irradiation was carried out in sodium bisulfite solution buffered at pH 9 or in sodium sulfite aqueous unbuffered solution (Fig. 2).

Purine nucleosides and cytidine were completely unaffected under these conditions.

In preparation, a 2×10^{-3} *M* of thymidine was irradiated with 500 W of a low-pressure mercury lamp (intensity of 10.5 W at 2537 Å) under the identical conditions. Irradiation for 60 min led to 98% disappearance of the uv absorbance at 267 nm. After filtration through a column of Amberlite IRC-50 (H^+ form) the reaction mixture was lyophilized. The amorphous residure showed two spots on tlc whose one was identical with that of dihydrothymidine. Because of difficulty of separation the mixture was hydrolyzed with 0.1 *N* hydrochloric acid, and the reaction mixture was chromatographed over a silica gel column to afford dihydrothymine and 2-oxo-4-methoxy-5-methylhexahydropyrimidine[2], mp 173°. Similarly, uridine was irradiated until 95% disappearance of the uv absorbance at 262 nm under the same conditions followed by hydrolysis of the photolyzate to give dihydrouracil as a main product.

In the past few years, a number of workers[3-9] have observed that bisultite ion in moderate concentrations added across the 5,6-double bond of cytidine and uridine. On the contrary, in these experimental conditions bisulfite exhibited no obserbable addition to the 5,6-double bond of pyrimidine nucleosides during 8 hr in the dark.

One of the principal advantages of this photoreduction is that reagents show

the selective photoreduction on the 5,6-double bond of uridine or thymidine without affecting base-sensitive dihydropyrimidine nucleus[2,10,11] formed.

Applications of this method to polynucleotides are now under investigation.

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