Tetrahedron Letters No. 19, pp 1619 - 1622, 1978. 0040-4039/78/0501-1619. $02.00/0.

PHOTOCHEMICALLY INDUCED REACTION OF 1,N^4-DIMETHYLCYTOSINE WITH ISOPROPANOL

Kieran I. Ekpenyong, Rich B. Meyer Jr., and Martin D. Shetlar*

Department of Pharmaceutical Chemistry

School of Pharmacy

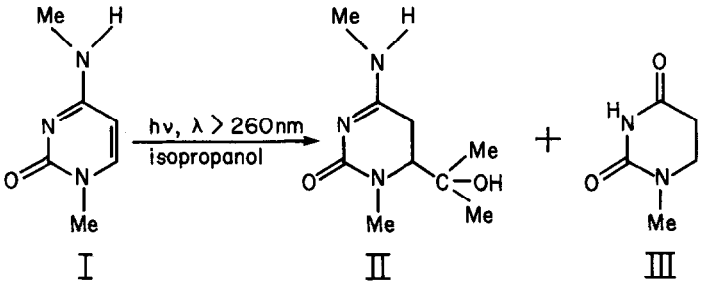
University of California

San Francisco, California 94143.

(Received in USA 24 January 1978; received in UK for publication 23 March 1978)

Summary. Photoexcited 1,N^4-dimethylcytosine (I) adds isopropanol to form 5,6-dihydro-1,N^4-dimethyl-6-(2-hydroxy-2-propyl)cytosine (II) as the major product; a small amount of 5,6-dihydro-1-methyluracil (III) is formed as well.

The photochemistry of cytosine and its derivatives is an area of significant importance for understanding the photoreactivity of nucleic acids. The area has, however, been somewhat neglected as compared to the photochemistry of the other four heterocyclic bases commonly occurring in nucleic acids. This is probably because of the pronounced tendency of photoproducts to deaminate, particularly those products that are saturated at the 5,6 bond. We report here the isolation and characterization of a relatively stable photoadduct of 1,N^4-dimethylcytosine (I) with isopropanol, namely 5,6-dihydro-1,N^4-dimethyl-6-(2-hydroxy-2-propyl)cytosine (II). This is evidently the first reported isolation and characterization of a cytosine photoadduct with an alcohol. It is interesting to note that the side chains of threonine and serine are alcoholic in nature. These two amino acids are found in proteins in intimate association with DNA and RNA in living cells (e.g. chromosomal and ribosomal proteins) Hydroxyl groups attached to carbon are also present in the ribose moieties in the RNA backbone. Thus knowledge of the photochemical reactivity of cytosine and its derivatives toward alcohols may be useful in helping achieve an understanding of the photochemistry of RNA and of nucleic-acid-protein systems.



I II III

Previous studies of the photochemistry of cytosine and its derivatives have emphasized the nature of photoreaction in aqueous media.[1] It has generally been found that at low concentrations the primary products result from addition of water across the 5,6 double bond to form 5,6-dihydro-6-hydroxycytosine. At higher concentrations of cytosine evidence for formation of cyclobutane type dimers as well has been reported. Yang and co-workers[2] have studied the photoreactivity of cytosine in aqueous solution with the amino acid cysteine and reported 5,6-dihydrouracil as the predominant product; presumably this compound arises from deamination of 5,6-dihydrocytosine. Several studies have reported following the photoreaction of cytosine and its derivatives in aqueous alcoholic solution using ultraviolet spectroscopy as a monitor. Wierzchowski and Shugar[3] reported the absorption maxima of the photo-reaction mixtures of various cytosine derivatives in aqueous ethanol. Helene and Douzou[4] found that cytosine formed products that were stable at room temperature in aqueous methanol, ethanol and butanol, but labile at higher temperatures. In neither of these studies were products isolated.

Solutions of I[5] (7.2×10^{-3} M in Mallinkdrodt spectroscopic grade isopropanol) were deoxygenated with prepurified nitrogen for five minutes and irradiated at $\lambda > 260$ nm with a Corex filtered Hanovia 450 watt pressure Hg lamp. (Undeoxygenated solutions were later found to undergo photoreaction at about the same rate.) Reaction progress was followed by ultraviolet absorption measurement. Reaction was allowed to proceed for about ten hours, which resulted in disappearance of about 50% of the intensity at the principle UV absorption maximum of I. (This corresponds to about 50% conversion to photoproducts.) Photoproducts were isolated by high pressure liquid chromatography on either Bio-Sil A or on a Whatman Partisil PXS 4.6 mm x 250 mm column using heptane-isopropanol-methanol (150:100:50) as the eluent. Photoproducts were purified, when necessary, by rechromatography on the same column.

The photoproducts of II and III were obtained as viscous oils which solidified on standing at room temperature. NMR evidence indicates that II is stable for only a few days in the solid state at room temperature. It is found to be stable for at least several weeks in chloroform at room temperature. The product II was formed in 75% yield while III was formed in 11% yield, based on total photoproducts collected from the chromatographic column. The remainder was minor amount of unidentified materials which may have come from decomposition of II.

The structures of the photoproducts were determined by application of IR, UV, NMR, and mass spectrometry. The IR spectra of II, run in chloroform on a Perkin-Elmer 457 Spectrophotometer, showed a broad absorption peak centered around 3400 cm^{-1}, corresponding to the OH-stretching vibration. The spectrum also showed strong absorption bands in the region between 1550-1750 cm^{-1} which corresponds to the carbonyl and amide region. Similar bands in the region between 1400-1640 cm^{-1} were found for III. The UV spectra were run in distilled water, buffered at pH 7, on a Varian 118C spectrophotometer. The compound II showed a λ_{max} at 249.0 nm (ε= 5290) and λ_{min} at 232.4 nm (ε = 3750) while III showed no UV absorption maximum above 200 nm, but did show end absorption rising from about 240 nm toward shorter wavelengths. The absorption maximum of II is in reasonable agreement with that found by Brown and Hewlins[6] for various dihydrocytosines. The FT-NMR of the products was run on a Varian XL-100 in $CDCl_3$ and gave the following results: II[δ 1.25, doublet, 6H(-C($\underline{CH}_3$)$_2$OH); δ 3.14, singlet, 3H (1-N-$\underline{CH}_3$); δ 2.98; singlet, 3H ($\underline{N}^4$ - $\underline{CH}_3$); δ 2.63, 2H, unresolved complex multiplet (5-$\underline{CH}_2$); δ 3.4, 1H, unresolved complex multiplet (6-$\underline{CH}$)]; III [δ 3.06, singlet, 3H, (1-N-$\underline{CH}_3$); δ 2.65, 2H, triplet (J = 7), 5-$\underline{CH}_2$; δ 3.4, 2H, triplet (J = 7), 6-$\underline{CH}_2$]. The NMR spectrum of III was identical with that of an authentic sample of 5,6-dihydro-1-methyluracil obtained from Vega-Fox Biochemicals. Low resolution mass spectrometry gave a molecular weight of 199 for II and 128 for III while high resolution chemical ionization mass spectrometry indicated a peak mass of 199.1320 for II which is consistent with a molecular formula of $C_9H_{17}N_3O_2$.

The site of attachment of the hydroxyisopropyl group to the cytosine ring was determined by study of the NMR spectra of II isolated from photolysis of 5-d-1,$\underline{N}^4$-dimethylcytosine in isopropanol. The product from this reaction showed a broadened one proton doublet in the AB part of the ABX multiplet located at δ 2.63. The 5-d-1,$\underline{N}^4$-dimethylcytosine was obtained by first synthesizing uracil deuterated at the 5-position by the method of Santi *et al.*[7]; the cytosine derivative was then prepared as in compound I.[5] The NMR of the cytosine showed nearly 100% substitution of deuterium at the 5-position.

Preliminary results indicate that the reaction is general; both 1-methylcytosine and 1,N^4,N^4-trimethylcytosine react with isopropanol to give adducts analogous to II while 1,N^4,N^4-trimethylcytosine reacts with ethanol to give an adduct of similar type in an acetone photosensitized reaction.

Research support from NIH Grants GM 23526 and RR-00892-01A1 and from the Patent Fund of the University of California is gratefully acknowledged.

References

1. (a) A. D. McLaren, and D. Shugar, Photochemistry of Proteins and Nucleic Acids, McMillan New York, 1964; (b) Photochemistry and Photobiology of Nucleic Acids, Vol. I and II, Academic Press, New York, 1976 (S. Y. Wang, Ed.).
2. N. C. Yang, R. Okazaki, and F. Liu, J. Chem. Soc. Chem. Commun. 462 (1974).
3. K. L. Wierzchowski and D. Shugar, in Progress in Photobiology: Proceedings of the Third International Congress on Photobiology, Copenhagen, B. C. Christensen and B. Buchmann, Ed., Elsevier, New York, 1960, p. 606.
4. C. Hélène, and P. Douzou, C. R. Acad. Sci. Paris 258, 196 (1964).
5. (a) G. E. Hilbert and T. B. Johnson, J. Amer. Chem. Soc. 52, 1152, 2001 (1930); (b) G. W. Kenner, C. B. Reese, and A. R. Todd, J. Chem. Soc. 855 (1955).
6. D. M. Brown and J. E. Hewlins, J. Chem. Soc. (C), 2050 (1968).
7. D. V. Santi, C. F. Brewer and D. Farber, J. Heterocyclic Chem. 7, 903 (1970).