REVERSIBLE PHOTOCHEMICAL TRANSFORMATION OF 5-FLUOROURACIL ANALOGUES AND POLY-5-FLUOROURIDYLIC ACID

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Replacement of thymine by 5-halogeno uracils in DNA generally provokes a marked increase in apparent radiation sensitivity to ultraviolet light. In addition the photoproducts are not susceptible to biological photoreactivation or other forms of reversibility.

During the course of an investigation on the photochemistry of 5-halogeno uracils, their glycosides, and oligo- and polyribonucleotides, it was observed that 5-fluorouracil (Fu) analogues gave rise to photoproducts capable of reverting to the parent substances in the dark. Since RNA is known to incorporate Fu in place of uracil (Gordon and Staehelin, 1958), while TMV-RNA containing incorporated Fu has likewise been reported to exhibit increased radiation sensitivity (Bećarević et al., 1963), we have examined in greater detail the nature of the photoproducts of various Fu derivatives, as well as poly-5-fluorouridylic acid (poly-FU).

The most suitable model compound for photoproduct formation and isolation was found to be 1,3-dimethyl-5-fluorouracil (DMFu), prepared by the methylation of

Fu with diazomethane, m.p. 128-130°, λ_{max} 2740 Å, ε_{max} 7.4 x 10^3. Irradiation of DMFu in aqueous medium at wavelengths to the red of 2600 Å resulted in almost quantitative conversion to a product which could be crystallized from ethanol and hexane, m.p. 130° (decomp.), λ_{max} 2200 Å, ε_{max} 5.0 x 10^3, and only end absorption at longer wavelengths.

The nature of this primary photoproduct was readily ascertained from the following facts: (a) the photochemical reaction was first order and the rate was concentration independent over a 10-fold range of concentration; (b) the absorption spectrum of the photoproduct indicated saturation of the 5,6 bond, since it was similar to the spectrum of dihydro-dimethyluracil (Janion and Shugar, 1960); (c) the infrared spectrum demonstrated the complete absence of the 1656 cm^{-1} C=C band of DMFu, and the appearance of an -OH band at 3580 cm^{-1} which underwent the expected characteristic shift on deuteration, while the original C-F frequency was essentially unchanged; (d) on heating the photoproduct in 1N HCl for 1 hour at 100°C, it reverted quantitatively to DMFu, demonstrated both spectrally and by chromatography in several solvent systems; furthermore, when the photoproduct was heated to 130° in a melting point capillary, the liberation of water vapour was observed and the resulting decomposition product exhibited the UV absorption spectrum of DMFu; (e) elementary microanalysis of the photoproduct gave C - 40.54%, H - 6.09%, N - 15.37%, corresponding to DMFu·H_2O.

The photoproduct must therefore be 1,3-dimethyl-5(6)-hydro-6(5)-hydroxy-5-fluorouracil. Although remarkably stable in both neutral and acid medium (see above) as compared to photohydrated uracil derivatives (Wierzchowski and Shugar, 1959), it is labile in alkaline medium, undergoing conversion to one or more as yet unidentified products.

On irradiation at wavelengths to the "blue" of 2600 Å, hydration of the 5,6 bond was still the principal reaction; but at these shorter wavelengths there was an additional photochemical transformation which was shown to be due to the effect of the shorter wavelengths on the primary, hydrated photoproduct. This secondary photochemical reaction, as well as the alkaline lability, of the primary hydrated photoproduct are strikingly similar to the previously observed behaviour of the bromination products of thymine derivatives (Barszcz et al., 1963).

The same photochemical transformations were exhibited by 5-fluorouridine, 5-fluorodeoxyuridine, 5-fluorouridine-5'-phosphate. Particularly interesting from a biological standpoint was the observation that the hydration reaction was the main, if not the only, primary photochemical transformation undergone by the Fu residues in poly-FU. It follows that TMV and TMV-RNA containing incorporated Fu should, following UV inactivation, be susceptible to some type of biological reactivation, e.g. photoreactivation. Furthermore, in view of the remarkable behaviour of irradiated poly-U in the *in vitro* amino acid incorporating system (Grossman,

1963), and of irradiated poly-U and poly-C in the DNA-controlled RNA polymerase system (Ono and Grossman, 1964), it should be of special interest to examine the function in both of these systems of irradiated poly-FU in which the photohydrated Fu residues are much more stable than the hydrated residues in irradiated poly-U and poly-C (Wierzchowski and Shugar, 1959, 1960).

Attention should also be drawn to the fact that the acid-catalyzed elimination of a water molecule from the photoproduct of DMFu exhibits the same isotope effect in heavy water as does the parallel elimination of a water molecule from the photoproducts of uracil derivatives. By contrast the isotope effect for the photohydration of DMFu is only 1.4 as compared to 2.0 for water addition to uracil derivatives (Shugar and Wierzchowski, 1957).

It remains to establish whether the hydroxyl of the water photoadduct is on the 5- or 6-position of the pyrimidine ring. It should be recalled that in photohydrated uracil analogues, the water hydroxyl is located on the 6-position (Moore and Thomson, 1955; Wang et al., 1956). The much greater stability of the photoproduct of DMFu as compared to that of DMu, and the fact that the C-F and -O-H infrared bands in DMFu·H_2O are intramolecularly unperturbed (and hence not involved in intramolecular hydrogen bonding), might be taken to imply the possibility of the location of the hydroxyl on the 5-position of DMFu·H_2O. However, a comparison of the properties of DMFu·H_2O with those of chemically synthesized 5-fluoro-6-hydroxyhydrouracil described by

Duschinsky et al. (1963) leaves little doubt but that the hydroxyl is, in fact, on the 6-position, so that the primary photoproduct of DMFu is 1,3-dimethyl-5-hydro-6-hydroxyhydro-5-fluorouracil. The infrared observations cited above consequently lead to the conclusion that the C-F and C-OH bonds in the photoproduct are trans with respect to each other. Attempts are under way by means of NMR and further infrared analyses to check this point.

Full details of the foregoing, together with additional data, and the findings obtained with other halogeno uracils and their glycosides and polymers, will be published elsewhere. We are indebted to Dr. R. Duschinsky of Hoffman-LaRoche for providing us with a transcript of his talk at the 145th National Meeting of the American Chemical Society; to Hoffman-LaRoche and the Cancer Chemotherapy National Service Center of the National Cencer Institute, Bethesda, for gifts of fluorouracil; and to Professor E. Lederer of the Institut de Chimie des Substances Naturelles, C.N.R.S., Gif-sur-Yvette, France for the elementary microanalyses.

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