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PHOTOCHEMISTRY OF URIDINE IN METHANOL. SUBSTITUENT AND CONCENTRATION EFFECTS

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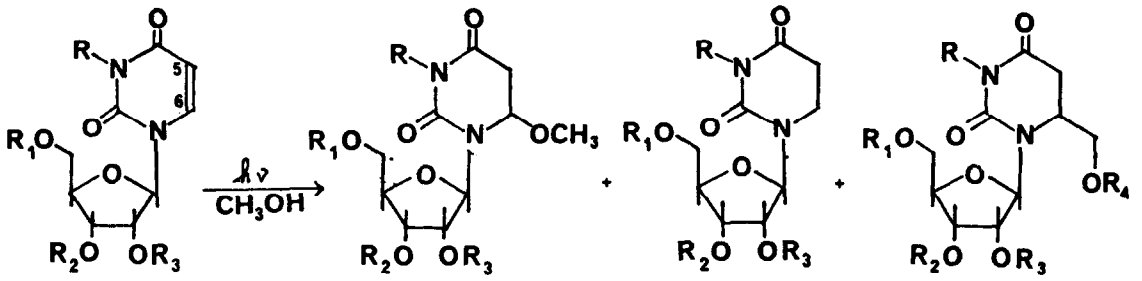
Photoreactivity of nucleic acid constituents is now being widely used to investigate interactions within a variety of biological systems (nucleic acid-protein[1] or nucleosidic substrate enzyme[2] complexes). Covalent bonding in the close vicinity of the association sites of these macromolecular complexes can be induced by irradiation (photoaffinity labelling). There are several instances where excited state uracil can be considered as the reactive species which might cross-link with amino-acids possessing a mercapto-, hydroxy, or amino-substituted side chain. This view is supported by recents reports on uracil photochemistry which have established that uracil, or its dimethyl derivative, undergoes photoaddition reactions in the presence of thiols[3,4], alcohols[5] and amines[6].

Because differences in the photoreactivity of 1,3-dimethyluracil and uridine 1a could be expected in view of substituent and conformation effects we were prompted to investigate the photochemical behaviour of several uridine derivatives in methanol. Such a photochemical study, carried out on nucleosidic substrates, should give valuable information (photoproduct structures, reaction mechanism...) concerning interaction requirements for bonding at the macromolecular level.



1 2 3 4

Irradiation of uridine 1a (and its derivatives 1b-1g) in methanolic solution[7] afforded three main photoproducts. Their relative yields and rates of formation are concentration dependent (Table 1).

Derivatives 2 which lose methanol upon warming to give back the parent uridine (thermal reversibility) are 5,6-dihydro-6-methoxyuridines. This has been proven for 2a, 2c, 2d and 2g by synthesis realized by an other route[8]. The 5,6-dihydrouridines 3 were identified with

TABLE 1 : PHOTOPRODUCT YIELD

Initial Nucleoside Concentration	10^{-4} M	10^{-3} M		
Photoproduct / Derivative	2*	2**	3**	4*
1a R = R_1 = R_2 = R_3 = H	58	30	18	22
1b R = R_2 = R_3 = H, R_1 = Ac	45	15	12	35
1c R = H, R_1 = R_2 = R_3 = Ac	26	7	25	55
1d R = R_1 = H, R_2, R_3 = $C(CH_3)_2$	53	20***	12	40
1e R = CH_3, R_1 = R_2 = R_3 = H	60	30	≤10	35
1f R = CH_3, R_1 = R_2 = R_3 = Ac	55	15	18	42
1g R = CH_3, R_1 = H, R_2,R_3=>$C(CH_3)_2$	62	36***	12	35

* Determined by thermal reversibility

** Givenin % photolyzed uridine. Yields of other minor photoproducts (presumably dimers) were not measured

*** In this case formation of O^6,5'-cyclo-5,6-dihydrouridine was detected. See following paper.

TABLE 2 : NMR DATA OF COUMPOUND 4g δ ppm

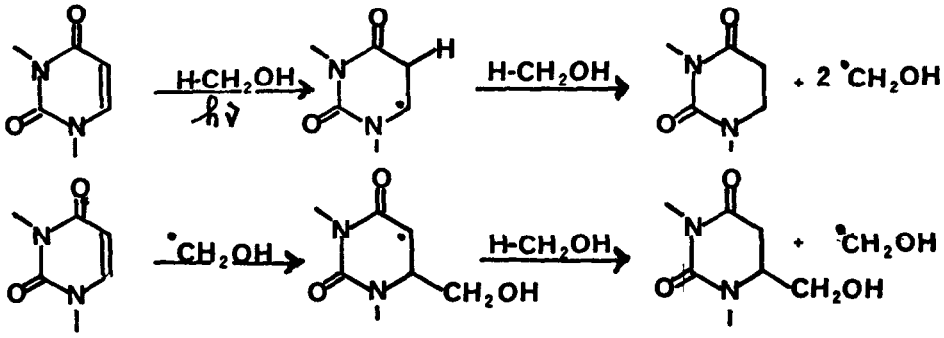
Epimer	H-1'	H-2', H-3'	H-4'	H-5' ; H-6 ; CH_2OH	N-CH_3	H-5
A	5.20 $J_{H-1', H-2'}$ = 2.9 Hz	~ 5	4.20	3.80 - 3.70	3.14	3.10-2.50 J_{gem}=16.7.Hz
B	5.38 $J_{H-1', H-2'}$ = 3.8 Hz	~ 5	4.20	3.80 - 3.70	3.15	3.10-2.60 J_{gem}=16.5 Hz

Solvent : $CDCl_3$

products resulting from usual hydrogenation procedures[9]. Compounds 4a-4f (R_4 = H) have been transformed into the same derivative 4g (R_4 = H) the two epimers(at C-6) of which could be separated by preparative TLC. Structure 4g (R_4 = H) was established on the basis of analytical and spectral data. Its mass spectrum shows that it is a methanol addition product. The absence of UV absorption above 230 nm is typical for a 5,6-dihydrouridine derivative. Acetylation of 4f (R_4 = H) gave a tetraacetate 4h (R_4 = Ac) suggesting that photoproduct 4 resulted from an hydroxymethylation process. In the NMR spectra of the two epimers 4g (R_4 = H) the signals

due to the two H-5 protons could be unambiguously assigned (Table 2). They give rise to characteristic AB patterns, part of an ABX system, centered at 2.75 ppm - an usual position for H-5 protons of dihydrouridine[10].

Thus, irradiation in methanol of the uridine derivatives herein studied (initial concentration 10^{-3} M) leads mainly to photoreduction. It is noteworthy that the formation of thermally stable photoproducts, through solvent coupling (hydroxymethylation), occurs exclusively at position C-6. When irradiation of derivatives 1a-1g was performed on a more dilute solution (initial concentration 10^{-4}M) the photoproducts detected were qualitatively the same. However, thermal reversibility measurements showed that the yield of solvent addition photoproducts 2 was higher (Table 1) while the rate of the reaction (measured by disappearance of UV absorption) was lower[11]. About 60 % of the UV absorption could be recovered in the case of uridine 1a and its N-3-methyl derivatives 1e, 1f and 1g. Unexpectedly, substitution on the ribose moiety (compounds 1b, 1c and 1d) inhibited the formation of the 6-methoxy compounds 2. Since the formation of reductive type photoproducts was decreased in the presence of oxygen it could be inferred that they derive from a triplet excited state.



Scheme

To account for the above findings, concentration dependence of the yield and of the rate of formation of photoproducts 3 and 4, two mechanisms may be suggested. In one case an excimer would be involved the formation and/or the reactivity of which might depend of uridine substitution. The other possibility is a chain reaction as depicted in the scheme. This second alternative for which there are precedents in the photochemistry of conjugated enone systems[12] seems the most reasonable in the present case[13]. The effect of ribose substitution could be to enhance either the triplet yield and/or the reactivity of the pyrimidinone towards hydroxymethyl radicals.

In conclusion, during photoaffinity labelling processes the most likely photoproducts to be formed are those which have structure 2. However reductive type products 4 could be produced by chemical sensitization, that is, through interaction of a ground state uracil with an hydroxyalkyl radical generated by a vicinal excited uracil or a sensitizer[15]. In the former case a specific spatial arrangement of the reactive species should be necessary[16].

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7 - 10^{-3}M(or 10^{-4}M) uridine derivatives solutions were irradiated under nitrogen atmosphere in a quartz vessel with low pressure Hg lamps (Hanau TQ 15-32) at room temperature. With higher initial concentration (over 10^{-2}M) dimerisation became preponderant.

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11 - For a 10 fold concentration increase (10^{-4} M → 10^{-3} M) the acceleration factor is ~1.4 ± 0.3. The inaccuracy results from difficulty to measure the initial rate in the case of dilute solutions.

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13 - At this stage it seems that a cage recombination mechanism is improbable since the formation of 5,6-dihydrouridines, which to some extent is a measure of cage escape, is always high compared to hydroxymethylation even in dilute solution. Interestingly we have shown that 5-(2-hydroxypropyl)-uracil does not undergo photocyclization[14]

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15 - J. Sperling and A. Havron, Biochemistry, (1976), 15, 1489 and references given therein

16 - We thank M. G. Henry for skillful technical assistance