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SPONTANEOUS AND LIGHT INDUCED HYDRATION OF PYRIMIDINES

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SUMMARY: Nmr spectral changes showed that 4-methoxy-1-methyl-2-oxopyrimidine in 2 M or 8 M D_2SO_4 gradually converts to 1-methyluracil and also C(5)-deuterium exchange was observed. While the rates of the former reaction are not influenced by irradiation (254 nm), the rates of C(5)-deuterium exchange show a 2.3 - to 5-fold increase. These findings indicate the significance of protonated ground state species in both the dark and light induced hydration of pyrimidines. A general mechanism for this type of reaction is proposed and its relevance to the photochemistry of nucleic acids is discussed.

Since pyrimidine photohydrates may play a significant role as lethal and mutagenic agents in UV irradiated nucleic acids (1), it becomes important to understand the chemical mechanism involved in the photoaddition of water to pyrimidine compounds. We wish to report observations on model pyrimidine systems demonstrating both light induced and spontaneous water addition which together suggests a possible general mechanism for this process.

The nmr spectra of 4-methoxy-1-methyl-2-oxopyrimidine (I) (2) and 1-methyluracil (II) (3) in 8 M D_2SO_4 are shown in Fig. 1A and 1B, respectively, with the signals specified. When I is allowed to stand at pD 1 in D_2O, its nmr signals gradually decrease with the appearance, and subsequent increase, of the signals for II.

The relative peak areas of C(5)H, C(6)H, and N(1)CH_3 for II should

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DISCLAIMER

be 1:1:3; however, the resulting areas are 1:1:6. This additional peak area is probably due to the signal for CH_3OD which has an identical chemical shift as $N(1)CH_3$ for II. This is to be expected since an equivalent amount of CH_3OD would be released during the conversion of I to II.

A mechanism for this CH_3OH exchange reaction may be deduced by close examination of the effects of pH. The kinetics are first order for both the loss of I and the formation of II, as calculated from the respective nmr peak areas. The relative first order rate constants as a function of pH are summarized in the Table. Evidently, this dark reaction rate is markedly increased as the pH is decreased from 2 to 0. This observation may be correlated with a previously determined basic pK_a at 0.65 ± 0.05 (4) for I, and suggests that protonation of I greatly enhances its conversion to II. It is known that uracil and its derivatives with predominately diketo structures protonate on exocyclic carbonyls (4) but that cytosine derivatives, with electronic structures more closely resembling I, protonate at N(3) (5). Protonation of I would thus be expected to give III. A reasonable mechanism (pathway A) then involves 1,2-addition of D_2O at N(3)-C(4) yielding IV which should be unstable, reverting either to III or to II by the loss of CH_3OD.

It was anticipated that at pD 1, 1,4-addition of D_2O to III (pathway B) should also occur yielding V. Upon ene-amine tautomerization, V gives VI which then loses HOD to form the C(5)-deuterium

exchanged starting material VII (6). This exchange may also occur without actual hydration and such a reaction also requires enamine tautomerization (7). However, VII was not detected. This is readily explained by the relative rates which indicate that the fast formation of II from I should preclude observation of C(5)H-exchange via pathway B. Yet ir and nmr analyses of the final product, crystallized directly from the nmr samples, showed only 70% II with 30% C(5)-D II (compound VIII). The latter must be formed either from II or VII by pathways analagous to B or A, respectively. It was possible to demonstrate that C(5)H-exchange does indeed take place at a slow but finite rate with I in 8 M and II in 2 M or 8 M D_2SO_4 (see Table and Fig.). In 8 M D_2SO_4, the doubly cationic species of I may exist and could account for the substantially reduced rate of 1,2-addition, which permits observation of 1,4-addition and C(5)H-exchange. In summary, these results demonstrate that hydration occurs at N(3)-C(4) and possibly also at C(5)-C(6) bonds of pyrimidines without light.

As elucidation of the mechanism for photo-induced water addition to pyrimidine derivatives is of primary interest, the effect of UV irradiation on the relative rates of each process was also investigated (Table). It is apparent that UV irradiation has only minor effects on the rate of methanol release. This is contrasted to a 2.3- to 5-fold increase in C(5)-deuterium exchange for both I and II upon photolysis. Establishing a pathway

for dark C(5)-exchange does not necessarily imply that the photo-induced exchange proceeds by the same mechanism. Nevertheless, a mechanistic similarity is indicated by consideration of the following features: (a) Both light and dark reactions of I and II produce the same major products as seen by the results of nmr spectra. (b) The rates of both light and dark C(5)H-exchange with either I or II show similar pH effects. (c) If the light-induced exchange involves a water addition in a dark step, a model compound, not exhibiting exchange in the dark, should also show no light-induced exchange. 2,4-Dimethoxypyrimidine, whose pK_a is more acidic than -0.9 (8) and therefore would not be protonated in 8 M D_2SO_4, is such a molecule. Deuterium incorporation at C(5) is not observed either after prolonged irradiation or after standing. Only very slow release of methanol is apparent.

Assuming that dark water addition occurs as a second step in the light induced C(5)-D incorporation and that the initial result of light absorption is only the alteration of the electronic configuration, it follows that irradiation of III must produce a species more reactive toward water addition (9). Since IX is a minor resonance form of III and an excited state of IX (IX*) has been suggested as the reactive species in photohydration (10), either direct absorption by IX or absorption by III giving an excited structure resembling IX* would more efficiently add water yielding V. This conclusion may provide an important insight into a gener-

al mechanism of pyrimidine photohydration since derivatives of cytosine and 4-thiouracil show similar reactions under identical conditions. To gain further evidence of the existence of the tetrahedral intermediates (IV), a study of acid catalyzed ^{18}O exchanged with uracil derivatives shows that there is also efficient formation of IV, i.e. water addition to the C(4)-carbonyl of this pyrimidine (11).

If ionic species such as IX are favored in highly acidic media and the photohydration of pyrimidines in general goes through such intermediates, an immediate prediction is that the quantum yields of photohydration should be greatly increased at low pH. This prediction has been verified in our laboratory for several uracil derivatives and the results are reported in a companion paper (12).

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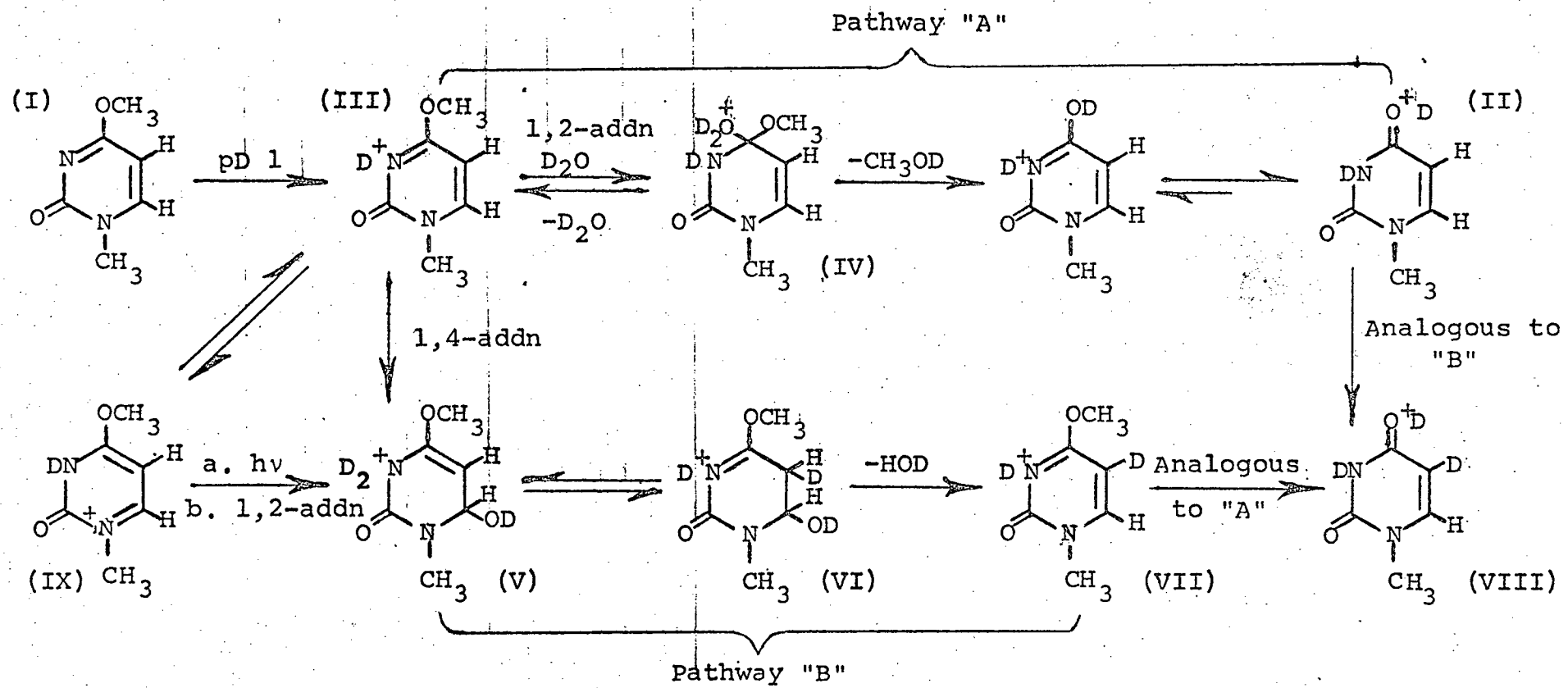
Proposed Mechanism for Spontaneous and Light Induced Hydration of Pyrimidine Derivatives



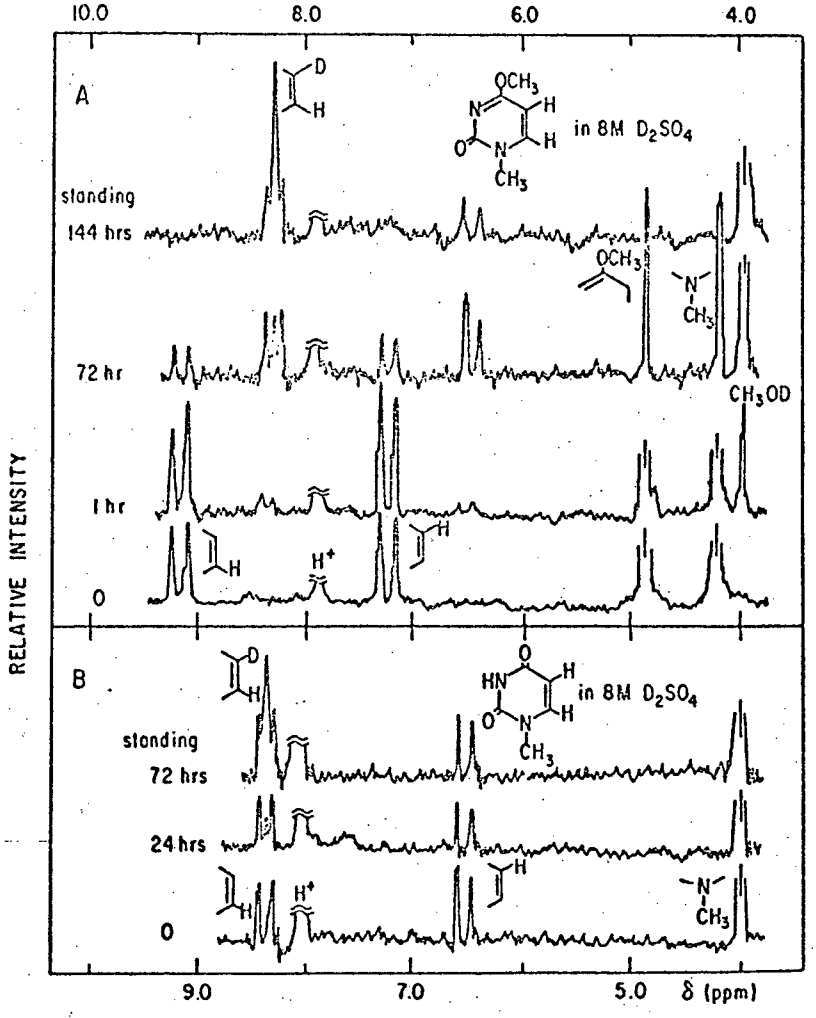

10.0
8.0
6.0
4.0
A
in 8M D_2SO_4
standing
144 hrs
72 hr
1 hr
0
CH_3OD
B
in 8M D_2SO_4
standing
72 hrs
24 hrs
0
9.0
7.0
5.0
δ (ppm)
RELATIVE INTENSITY

Table: Relative Rates of Methanol Loss and C(5)-deuterium Exchange of Pyrimidine Derivatives in Acidic Media

Reactant	pD	UV[a]	Relative First Order Rates	
			Methanol Loss	C(5)-d exchange
I	6	no	<1	<1
I	2	no	21	<1
I	1	no	367	--
I	0	no	475	--
I	-0.9	no	90	10
II	-0.3	no	--	10
II	-0.9	no	--	10
X	-0.9	no	<1	<1
I	6	yes	15	<1
I	2	yes	22	5
I	-0.9	yes	90	23[b]
II	-0.3	yes	--	21[b]
II	-0.9	yes	--	53[b]
X	-0.9	yes	2	<1

(a) The first order rates quoted for the UV irradiated samples are the best fit of the nmr peak areas plotted as a first order reaction and do not necessarily imply that the light induced reaction is truly first order.

(b) Corrected for the rate of dark C(5)-deuterium exchange assuming it to proceed independently of the light reaction.