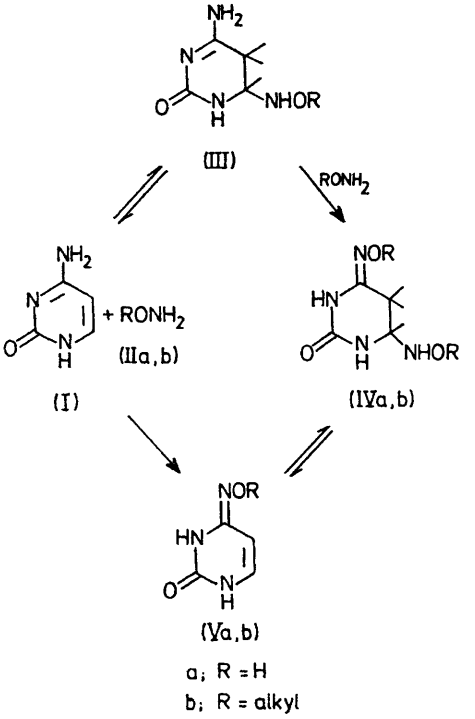
Mechanism of the Reaction of Cytosine with Hydroxylamines

By PETER M. SCHALKE and C. DENNIS HALL*

(*Department of Chemistry, King's College, Strand, London* WC2R 2LS)

Summary Kinetic and stereochemical studies are cited to support the proposal of a novel mechanism for the formation of 4-(alkoxyimino)-6-(alkoxyamino)tetrahydropyrimidin-2(1*H*)-ones from cytosine (I) and hydroxylamines (II).

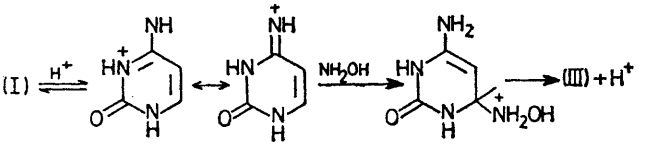
THE reaction of cytosine (I) with hydroxylamine (IIa) or *O*-alkylhydroxylamine (IIb) is known to give two products (IVa or b) and (Va or b) with the former predominating.[1,2]



SCHEME 1. Existing scheme for the reaction of cytosine with hydroxylamine or *O*-alkylhydroxylamine.

Previous mechanistic studies suggest that the principal pathway to (IVa, b) involves addition of hydroxylamine to the 5,6-double bond of (I) to give the undetectable intermediate (III) (Scheme 1).[3,4]

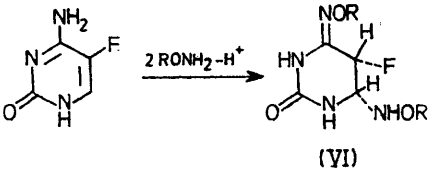
Our kinetic studies of the reaction of (I) with hydroxylamine (IIa) reveal: (i) that there is a rate maximum at pH *ca.* 6·0 (the pK_a of IIa);[5] (ii) that at constant pH (5·8) and constant ionic strength (2M) the reaction is first order in [(I)] and [(IIa)]; (iii) that there is a rate maximum at pD = 6·6 in D_2O and that k_H/k_D = 3·0† at the respective maxima under identical conditions of concentration (in IIa) and temperature. Hence the reaction is acid-catalysed and in terms of the existing mechanism must occur through nucleophilic attack of hydroxylamine on position 6 of cytosine (protonated at N-3)[6] followed by proton transfer from N to C (Scheme 2).



SCHEME 2. Details of acid-catalysed addition of (IIa) to C-6 of (I) for the existing mechanism.

The first order behaviour in hydroxylamine suggests that the rate-determining step of Scheme 1 is addition to the 5,6-double bond. Such a mechanism would be expected to show a rate maximum between the pK_a value of (I) and (IIa)[7,8] i.e. at pH *ca.* 5·2. Furthermore, the mechanism does not involve proton transfer in the rate-limiting step and might, in fact, be expected to display an inverse deuterium isotope effect.[9] Finally, nucleophilic addition of (IIa) to C-6 of protonated (I) is analogous to acid-catalysed addition to the β-position of an $\alpha\beta$-unsaturated imine, a reaction which should be non-stereospecific.

N.m.r. spectra (^{1}H and ^{2}H) of the product (IVb, R = CH_2Ph) from the addition of *O*-benzylhydroxylamine to (I) in D_2O show that one atom of deuterium is incorporated at C-5 and suggest that the reaction is stereospecific. More importantly however, addition of *O*-benzylhydroxylamine or *O*-isobutylhydroxylamine to 5-fluorocytosine confirms that addition to the 5,6-double bond to form (VI) is stereospecific, and is probably a *trans* addition. For



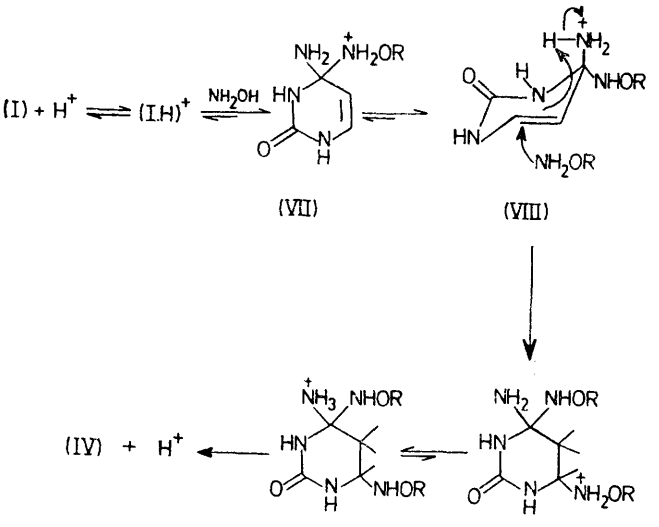
R = Bu^i, the *gem* H(5)-F coupling is 49 Hz and J_{H_6-F} = 18 Hz which is compatible with a *trans* disposition of H(6) and F‡ and hence a *trans* configuration for the NHOR group and H(5).[10] The *trans* addition has been suggested before,[11] the evidence being a value of $J_{H_5-H_6}$ = 2 Hz for a C-5 monodeuteriated compound of type (IV). However a coupling constant, $J_{H_5-H_6}$ = 1·5 Hz, was cited as evidence for *cis* addition to 5-methylcytosine[4] and analysis of several ABX systems[2] shows that *trans* addition cannot be proved unambiguously by ^{1}H n.m.r. spectra of compounds of type (IV). For (VI, R = Bu^i) $J_{H_5-H_6}$ = 2·6 Hz.

The most satisfactory mechanism to account for the kinetic and stereochemical facts is a fast, virtually irre-

† Corrected for the % deuterium (90%) in the reaction mixture.

‡ Incidentally, this also determines the predominant and possibly the only conformer of (VI) with H(6) and F in axial positions (see refs. 2 and 10).

versible, acid-catalysed addition of hydroxylamine to C-4§ to form (VII) followed by a fast proton transfer to (VIII).



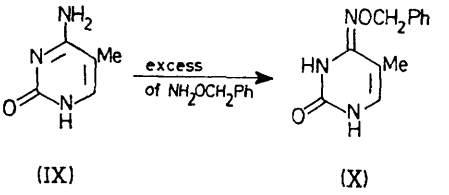
SCHEME 3. Proposed mechanism for the formation of (VI)

This is the critical intermediate for the next step, rate determining nucleophilic addition of NH_2OR to C-6 promoted by a synchronous intramolecular proton transfer from the $^+NH_3$ group of (VIII) to C-5 which enforces the *trans* addition to C(5)–C(6) and gives rise to the deuterium isotope effect (Scheme 3).

Intermediate (VIII) is also the source of the small quantity of substitution product (V) formed during the reaction of (I) with (II) especially at low concentrations of (II) or low pH where the rate of loss of NH_3 may compete successfully with attack of a second molecule of hydroxylamine. The intermediate has a 3,4-dihydropyrimidine structure which probably has a u.v. chromophore similar to that of cytosine itself[12] and its existence explains a number of puzzling preparative results.

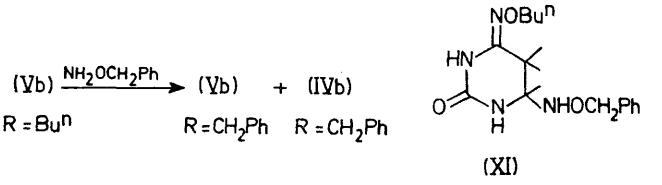
For instance, isolation of (Vb) from the reactions of (IIb) with (I) in D_2O revealed no deuterium at C-5. Since elimination of (IIb) from (IVb) at pH 6 (i) is very much slower than the reaction to form (IVb) and (ii) is non-stereospecific under acid-catalysed conditions,[11,13] (V) could only have appeared directly from (VIII).

Secondly, 5-methylcytosine (IX) reacts with (IIb, R = $PhCH_2$) to give only the substitution product (X). With *trans* addition, the methyl group would be *cis* to the second hydroxylamine molecule approaching C-6 and would therefore present a steric barrier in the rate-determining step. The same steric inhibition argument is applicable to the existing mechanism but does not explain why 6-methylcytosine forms derivatives of type (V) but not of type (IV).[8,14] The new proposals however, would view this as a consequence of a 1,3 interaction between the 6-methyl group and the $^+NH_3$ group in the transition state from intermediate (VIII) to product.



(IX) (X)

Finally the reaction of 4-n-butyloxyimino-uracil (Vb, R = Bu^n) with *O*-benzylhydroxylamine gives (Vb, R = $PhCH_2$) and (IVb, R = $PhCH_2$) but no (XI) despite the fact that structures of the latter type are obviously stable.[2]



(XI)

One must conclude that addition to the 5,6 double bond of cytosine or 4-oxyimino-uracils¶ (V) to form intermediates of type (III) is extremely unlikely and the alternative mechanism, involving (VIII) as the critical intermediate, provides a more plausible rationalisation of the existing facts. Whether or not the pH–rate profile represents a change in the rate-determining step remains to be confirmed but variations in the deuterium isotope effect over the pH range 4—9 suggest that such a change is likely.

We thank DAAD for support (to P.M.S.).

(*Received, 18th March* 1976; *Com.* 280.)

§ ^{13}C N.m.r. spectra reveal that this carbon atom is more electrophilic than C-6.[2]

¶ The reactions of 4-oxyimino-uracils with hydroxylamines are governed by the same kinetic laws as found for cytosine.

[1] J. H. Phillips and D. M. Brown, *Progr. Nucleic Acid Res.*, 1967, **7**, 349; D. M. Brown and P. Schell, *J. Mol. Biol.*, 1961, **3**, 709; N. K. Kochetkov, E. I. Budowsky, and R. P. Shibaeva, *Biochim. Biophys. Acta*, 1963, **68**, 496.
[2] P. M. Schalke and C. D. Hall, *J.C.S. Perkin I*, 1975, 2417.
[3] D. M. Brown and P. Schell, *J. Chem. Soc.*, 1965, 208; E. I. Budowsky, E. D. Sverdlov, R. P. Shibaeva, G. S. Monastyrskaya, and N. K. Kochetkov, *Biochim. Biophys. Acta*, 1971, **246**, 300.
[4] D. M. Brown and M. J. E. Hewlins, *J. Chem. Soc.*, 1968, 1922.
[5] This has been reported before: D. W. Verwoerd, H. Kohlhage, and W. Zillig, *Nature*, 1961, **192**, 1038.
[6] E. D. Becker, H. T. Miles, and R. B. Bradley, *J. Amer. Chem. Soc.*, 1965, **87**, 5575.
[7] W. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969.
[8] N. K. Kochetkov and E. I. Budowsky, *Progr. Nucleic Acid Res.*, 1969, **9**, 431.
[9] R. P. Bell and A. T. Kuhn, *Trans. Faraday Soc.*, 1963, **59**, 1789.
[10] A. Peake and L. F. Thomas, *Trans. Faraday Soc.*, 1966, **62**, 2980; L. D. Hall and D. L. Jones, *Canad. J. Chem.*, 1973, **51**, 2925. We are indebted to a referee for pointing out the significance of the latter paper and related papers to the stereochemical arguments.
[11] D. M. Brown and P. F. Coe, *Chem. Comm.*, 1970, 568.
[12] F. Sweet and J. D. Fissekis, *J. Amer. Chem. Soc.*, 1973, **95**, 8741.
[13] P. M. Schalke, unpublished results.
[14] C. Janion and D. Shugar, *Acta. Biochim. Polon.*, 1965, **12**, 337.