Stereochemistry of the Addition Reaction of Hydroxylamine and Methoxyamine with 1-Methylcytosine

By D. M. BROWN* and P. F. COE

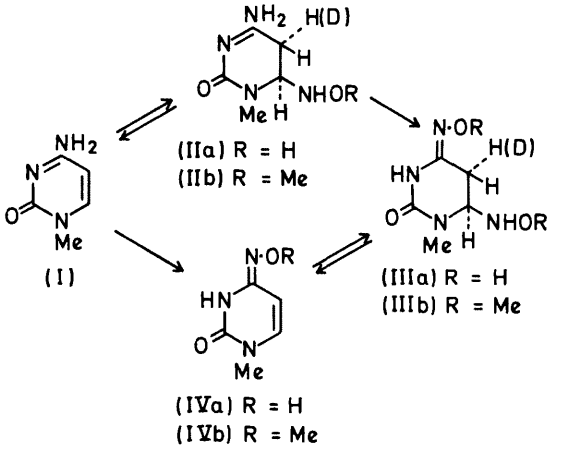
(*University Chemical Laboratories, Lensfield Road, Cambridge* CB2 1EW)

Summary Hydroxylamine and methoxyamine undergo *trans*-addition to the 5,6-double bond of 1-methylcytosine.

IT is known that hydroxylamine and methoxyamine react with cytosine and 1-substituted cytosines according to the Scheme.[1,2] Products (III) and (IV) arise by two distinct pathways, the latter by direct substitution and the former by addition across the 5,6-double bond to give (II) followed by rapid substitution to give the di-adduct (III).[3] (IV) and (III) can enter into an equilibrium under the reaction conditions but this is slow compared with the overall rate.

We treated 1-methylcytosine (I) with 3·0M-deuteriated solutions of hydroxylamine and methoxyamine at pD's 6·8 and 5·5 respectively, at 38°. The corresponding di-adducts (IIIa) and (IIIb) were isolated and purified.

On comparing the 100 Hz n.m.r. spectra in D_2O of these di-adducts with the corresponding di-adducts from aqueous solution it is clear that one deuteron has been introduced stereospecifically at C-5. The spectra for the non-deuteriated di-adducts are good first-order ABX systems. The two C-5 protons appear as an AB quartet further split by the C-6 proton with J_{5a5e} 15, J_{5a6e} 5, and J_{5e6e} *ca.* 2 Hz consistent with a half-chair conformation similar to dihydrouracils with the hydroxyamino- and methoxyamine-substituents axial.[4,5] The products from the deuteriated



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solutions show the disappearance of the C-5 axial proton and a di-equatorial coupling of *ca.* 2 Hz remains. Therefore, hydroxylamine and methoxyamine have added *trans* di-axially.

Equilibration of the monodeuteriated di-adduct (IIIa) in

3·0M-deuteriated hydroxylamine pD 7·2 at 70° gave a mixture containing (IVa) with complete loss of deuterium and without further incorporation of deuterium into the di-adduct. This is consistent with an all-*trans* *E*2 type elimination.

Treatment of the monodeuteriated di-adduct (IIIa) with 1N-HCl at 38° gave (IVa) quantitatively. About half of the C-5 protons in the product were replaced by deuterium. This suggests loss of the protonated hydroxyamino-group in a slow step followed by rapid non-specific loss of a proton or deuteron from C-5.

During the preparation of the monodeuteriated di-adduct (IIIa) there was no incorporation of deuterium into the starting material (I). This is of relevance to the suggested reversibility of the reaction (I) → (II).[6]

The mechanistic implications of these results will be discussed later. There are a growing number of examples of *trans*-addition to activated double bonds,[7] which include the recently reported addition of bisulphite ion to uridine and cytidine.[8]

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