Tetrahedron Letters,Vol.26,No.24,pp 2891-2894,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

REGIO- AND STEREOSELECTIVE ALKYLATION AT THE 3'-TERMINAL END OF RIBONUCLEOTIDES BY N-2-METHYL-9-HYDROXYELLIPTICINIUM ACETATE : AN ANTITUMOR AGENT

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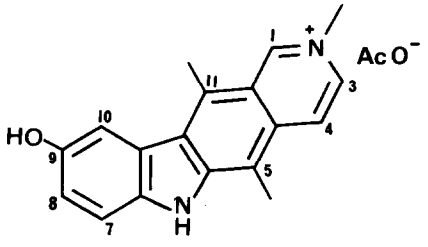
Abstract : The regio- and stereoselective alkylation at the3'-terminal end of ribonucleotides by N-2-methyl-9-hydroxyellipticinium acetate under oxidative conditions is reported.

N-2-methyl-9-hydroxyellipticinium acetate (NSC 264137, 9-OH-NME$^+$) 1, a derivative of plant alkaloid ellipticine[1], exhibits high cytotoxic activity[2] and has recently been used in the chemotherapy of cancer[3,4].

The in vivo mechanism of action of 9-OH-NME$^+$ is still controversial. However, intercalation of 9-OH-NME$^+$ between DNA base pairs has long been considered as the main explanation for its antitumoral activity[5,6]. Other proposals remain attractive[7,8]. Recently during the course of our reinvestigation of the oxidative alkylation of 9-OH-NME$^+$by ribonucleosides[7], we have established regio- and stereoselective formation of a ketalic linkage at position 10 of 1 by the 2' and 3'-OH groups of ribonucleosides[8]. These results designate RNAs as possible targets for 1.



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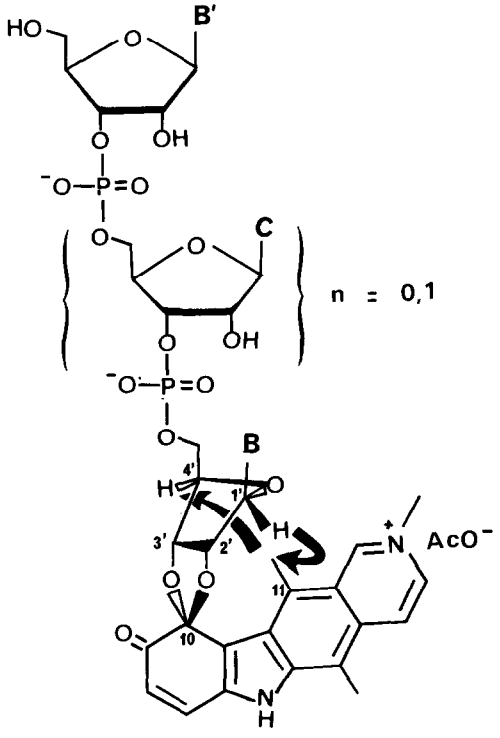
To test this hypothesis on more elaborated models, we initiated a detailed study of the reaction of ribunocleotideswith 1 under the same conditions reported earlier[7]: the results of this study are presented in this paper. The remarkable regio- and stereoselectivity at carbon 10 in 1 was thought to be related to the possible template, obtained by the stacking of the nucleotidic base and the ellipticinium ring prior to oxidation. This study also allows one to evaluate the influence of stacking at the end versus insertion between consecutive bases on the mode and the sites of the reaction.

Reaction of 9-OH-NME$^+$ (1 eq) with various ribonucleotides (5 eq) under oxidative conditions (horse radish peroxidase-H_2O_2) in a phosphate buffer (pH = 7.02, 2 x 10^{-5}M) gave adducts 2-4 in good yields (70-90%) within 10 mn. The adduct formation was studied by reverse phase HPLC (C-18 Bondapak column, 254 n.m. UV detector, $NH_4^+AcO^-$ buffer, 1 x 10^{-3} M in MeOH/H_2O (50/50), adjusted to pH 4.5 by adding glacial AcOH).

The structures of the adducts 2-4 were conclusively assigned on the basis of their spectroscopic data (U.V., FAB-MS, ^{1}H NMR), which were in excellent agreement with our previous results with ribonucleosides[8].



2 B' = C, B = A, n = 0

3 B' = A, B = A, n = 0

4 B' = A, B = A, n = 1

5 B' = G, B = C, n = 0

6 B' = G, B = U, n = 0

7 B' = U, B = G, n = 0

Figure 1

^{1}H NMR (400 MHz) characteristics of the adducts revealed the following facts and support the assigned structure :

The 3'-ribose end of the adducts were readily identified in the ^{1}H NMR subspectra (400 MHz), CD_3CO_2D/D_2O, due to the absence of ^{31}P coupling of the 3'-proton present in this ring. An observed downfield shift of the protons in the 3'-ribose end of the ribonucleotides in the adducts for example in 2

compared with the parent compound CpA (A_1, 6.61 versus 6.22, A_2, 6.25 versus 4.79, A_3, 5.85 versus 4.64 and A_4, 5.10 versus 4.34) suggested adduct formation at this end.

The doublets present at 7.6-7.8 and 6.15-6.25 for H_7 and H_8 respectively are characteristics of the unsaturated ketone present in the adducts.

The small values for both $^3J\ H_1,-\ H_2$, and $^3J\ H_3,-H_4$, couplings indicate an energetically unfavourable O'-exo conformation[9], stabilised by a ketalic linkage.

The observation of a strong NOE-effect between the 11-methyl group and both the H_1, and H_4, protons of the 3'-end of the ribose moiety supports the absolute configuration at carbon 10 as shown in Figure 1.

This study was then extended to various other diribonucleotides (GpC, GpU, UpG), which also showed formation of a single adduct. The U.V. and mass spectra of these adducts 5-7 compared well with the former fully characterised adducts (2-4). Therefore, the spiro structures 5-7 are proposed for these compounds.

It was also found that ribonucleotides having a purine base at the 3'-terminal end react faster than ribonucleotides having a pyrimidine base at this position. In order to confirm this observation, a competitive reaction of 9-OH-NME$^+$ (1 eq) with UpG (5 eq) and GpU (5 eq) in one pot was carried out. On comparison with the reference adducts on HPLC, it was observed that 9-OH-NME$^+$ selectively reacted with UpG to yield 7 while none of the adduct 6 was detected.

In conclusion, the formation of a stable ketalic linkage at the 10 position of 9-OH-NME$^+$ by the cis-hydroxyl group present at the 3'-end of various ribonucleotides supports the hypothesis that 9-OH-NME$^+$ could alkylate at the 3'-end of t-RNA to stop the formation of aminoacyl t-RNA or at the "cap" present at 5'-end of m-RNA or poly A tail present in m-RNA and subsequently could inhibit the biosynthesis of proteins.

The regio-, stereoselectivity of the reaction and the absence of other possible adducts, illustrated that stacking at the 3'-terminal end of ribonucleotides could be the decisive factor of this reaction compared with other possible interactions such as insertion between bases or ionic interactions.

Acknowledgement :

We thank Roussel-Uclaf for a postdoctoral research fellowship to Vinod K. Kansal and PIRMED (CNRS) for financial support.

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(Received in France 20 April 1985)