Tetrahedron Letters,Vol.25,No.22,pp 2351-2354,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

REINVESTIGATION OF THE REACTION OF N-2-METHYL-9-HYDROXYELLIPTICINIUM ACETATE WITH ADENOSINE AND METHANOL.

V.K.Kansal,S.Funakoshi,P.Mangeney and P.Potier*.
B.Gillet,E.Guittet and J.Y.Lallemand (Laboratoire de RMN).

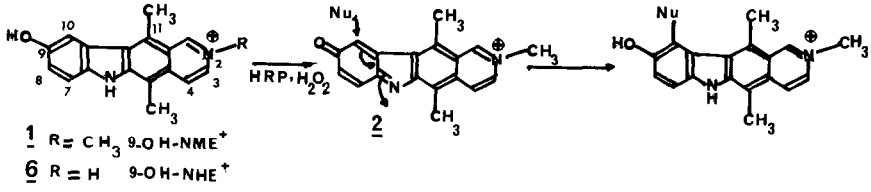
Institut de Chimie des Substances Naturelles,
Centre National de la Recherche Scientifique,
91190-Gif sur Yvette-FRANCE

Abstract: Reaction of the quinone imine obtained by oxidation of 9-hydroxyellipticine derivatives with adenosine and methanol has been reinvestigated and the structures of the adducts established.

N-2-Methyl-9-hydroxyellipticinium acetate (NSC 264137, 9-OH-NME$^+$) 1 has been shown to possess antitumoral activity and is currently under clinical trial in humans (1) (it is manufactured under the trade name Celliptium by SANOFI, in FRANCE).

The intercalation property of 9-OH-NME$^+$ with DNA (2) is probably not sufficient to explain its antimitotic activity. Therefore, in order to understand the mechanism of action, attention has been directed towards the study of its chemical properties (3-6).

Reaction of 9-OH-NME$^+$ with H_2O_2 in presence of horse radish peroxydase (HRP) gave an electrophilic quinone-imine 2, which undergoes addition of various nucleophiles such as pyridine, methanol, thiols, amino- acids and nucleosides (4-6) selectively at position 10. It is believed that, in vivo, quinone-imine 2, generated by the oxidation of 9-OH-NME$^+$, may serve as a strong alkylating species(scheme 1).

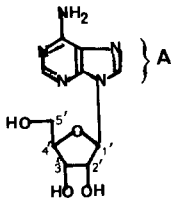


Scheme 1.

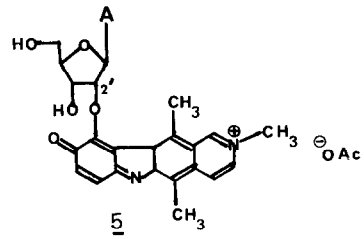
Isolation of 9-OH-NME$^+$-10-S-glutathione conjugate, as a bile excretion metabolite in rats treated with 1, possibly supports this hypothesis (7).

In order to establish the mode of action of 9-OH-NME$^+$ 1 in vivo, adduct 4 of adenosine 3 with quinone-imine 2 was prepared as described by Meunier et al. (5). The very small values observed in the proton NMR spectra of 4 for the JH3'-H4' and the JH1'-H2' coupling constants indicate that the ribose ring adopts an energetically unfavorable O'-exo conformation (8),

which did not support the proposed structure 5 (5).



3



5

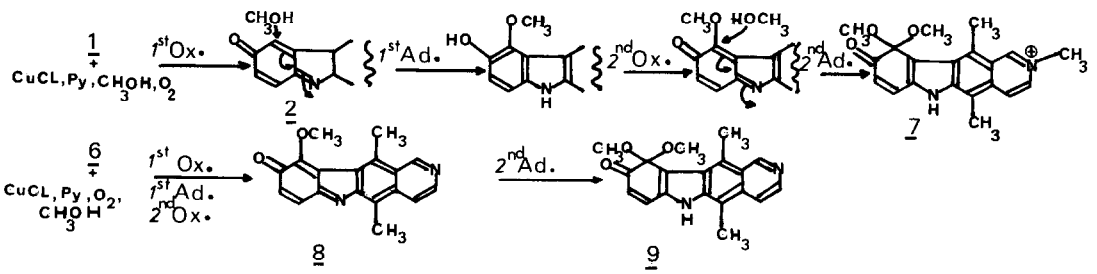
The correct structure 4 was established by ^{13}C and two-dimensional cross-relaxation correlated NMR spectroscopy (NOESY) (9).

In the ^{13}C spectrum of 4 (CD3COOD), a signal at 198.56 ppm is attributed to the carbonyle carbon of a conjugated ketone rather than to that of a quinone (~185 ppm) (10,11). Carbons 2' and 3' of the ribose ring are found respectively at 89.50 and 86.62 ppm and both are involved in an ether bond. A signal at 107.46 ppm is attributed to an quaternary ketalic carbon. The remaining signals in the ^{13}C spectrum (12) are consistent with the structure 4 that we propose for the adduct.

The absolute configuration of carbon 10 was established by two-dimensional NOESY spectroscopy. Clear correlations between the methyl at position 11 and the H4' and H1' of the ribose ring are visible in figure 1 and indicate that ribose and methyl 11 are close to each other, such a situation is only compatible with the configuration represented by formula 4 in figure 1.

The structure of MeOH 9-OH-NME$^+$ adduct was also investigated. Adducts of methanol with 1 and 9-hydroxyellipticinium acetate (9-OH-NHE$^+$) 6 were prepared using CuCl-pyridine in methanol in presence of oxygen (4,6). It was observed that 9-OH-NME$^+$ 1 yielded exclusively a dimethoxy adduct 7 , which had previously been wrongly assigned as a monomethoxy adduct (4,6), 9-OH-NHE$^+$ 6 gave a mixture of a mono 8 and a dimethoxy adduct 9 in a 1/5 ratio. Structures of 7 , 8 and 9 were elucidated by spectroscopic methods (12). In the proton spectra, the chemical shifts of the methoxy signals were found at 4.21 ppm (3H) for the monomethoxy adduct 8 (CDCl3) and at 3.36 ppm and 3.29 ppm (6H) respectively for the dimethoxy adducts 7 ((CD3)2CO) and 9 (CDCl3), all in close agreement with reported values for analogous systems (10,11). The indolic amino protons of 7 and 9 were found respectively at 8.89 ppm and 9.34 ppm.

To account for the formation of the ketal, a two-step mechanism, consisting of two successive oxidations and Michael additions can be proposed(scheme 2).



Scheme 2.

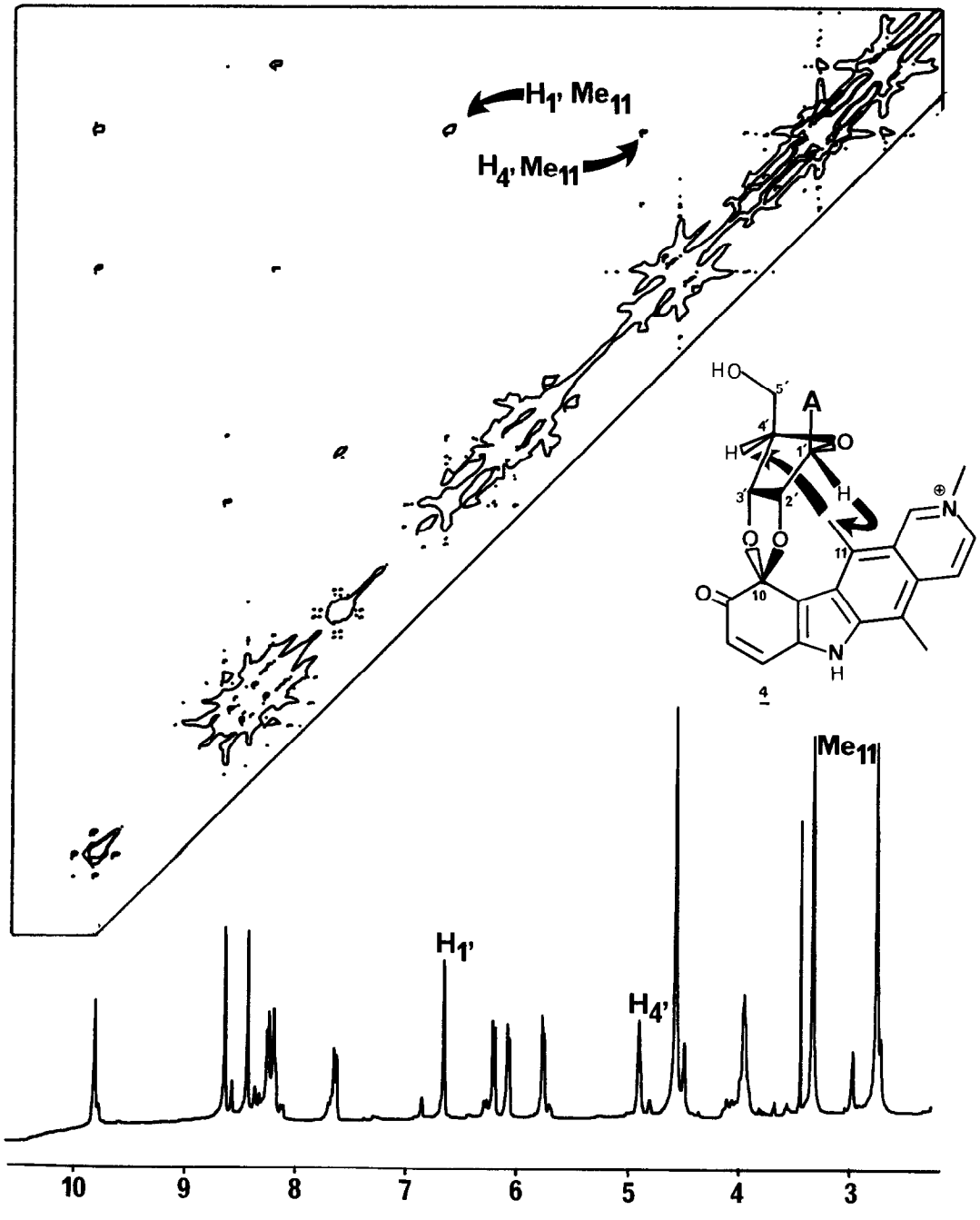


Figure 1. Two-dimensional NOE spectrum of 4 (.3 s mixing time) with the one dimensional ^{1}H spectrum across the bottom.

The reaction with adenosine is fast (10 minutes), regiospecific, stereospecific and highly selective (adenosine reacts faster than water, the solvent).

The reasons for this stereochemical control are currently under investigation.

Among the various possible biological consequences of this easy formation of ketalic nucleoside adducts one can imagine that in vivo 9-OH-NME^{+} reacts, after oxidation, with the 3' terminal nucleotide of mRNA and tRNA or with the 7-methylguanosine of the caps at the 5'end of eucaryotic mRNA, resulting in a subsequent inhibition of the early stages of protein biosynthesis.

Aknowledgements. The authors would like to thank M.T.Adeline for her help in the HPLC purifications.

References

1a)C.Paoletti,J.B.Le Pecq,N.Dat Xuong,P.Juret, H.Garnier, J.L.Amiel et J.Rouesse, Recent results in Cancer res. , 74 ,107(1980).

b)P.Juret,J.F.Heron,J.E.Couette,T.Delozier and J.Y.Le Talaer , Cancer Treat. rep. , 66 , 1909(1982).

2)J.B.Le Pecq,N.Dat - Xuong,C.Gosse,C.Paoletti, Proc.Natl.Acad.Sci.USA , 71 ,5078 (1974).

3)C.Auclair and C.Paoletti, J.Med.Chem. , 24 ,289(1981).

4)G.Meunier,B.Meunier,C.Auclair,J.Bernadou and C.Paoletti, Tetrahedron Letters ,365 (1983).

5)B.Meunier,C.Auclair,J.Bernadou,G.Meunier,M.Maftouh,S.Cros,B.Monsanat and C.Paoletti in "Structure-relationships of antitumor agents" ,Edit.D.N.Reinhoudt,T.A.Connors, H.M.Pinedo, K.W. Van De Poll,C.Martinus and N.Jhoff,La Hague,1983,p 149.

6)J.Bernadou,G.Meunier,C.Paoletti and B.Meunier, J.Med.Chem. 26 ,574(1983).

7)M.Maftouh,G.Meunier,B.Dugue,B.Monsanat,B.Meunier and C.Paoletti, Xenobiotica 13, 303(1983).

8)G.G.Govil and R.V.Hosur in "Conformation of biological Molecules" ,Edit.P.Diehl, E.Fluck and R.Kosfeld,Berlin H eidelberg,1982,p 46.

9)S.Macura,Y.Huang and R.R.Ernst, Biochem.Biophys.Res. Commum. 90 ,305(1979).

10)D.R.Henton,K.Anderson,J.Manning and J.Swenton, J.Org.Chem. 56 ,34(1980).

11a)P.Joseph Nathan,D.Abramo Bruno and D.Ortega, Org.Magn.Reson. 15 ,311(1981).

b)A.Kubo,S.Nakahara,R.Iwata,K.Takahashi and T.Arai, Tetrahedron Letters , 21 3207(1980).

12)All compounds show satisfactory spectral data (IR,UV, ^{1}H and ^{13}C NMR).

(Received in France 15 March 1984)