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INTERACTION OF NUCLEOSIDES WITH ANTITUMOURAL AGENTS : 9-HYDROXY-N-2-METHYLELLIPTICINIUM ACETATE AND 9-HYDROXY-N-2-METHYLOLIVACINIUM ACETATE

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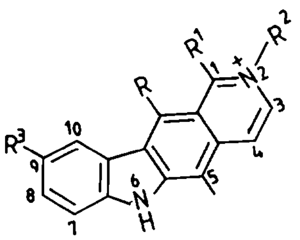
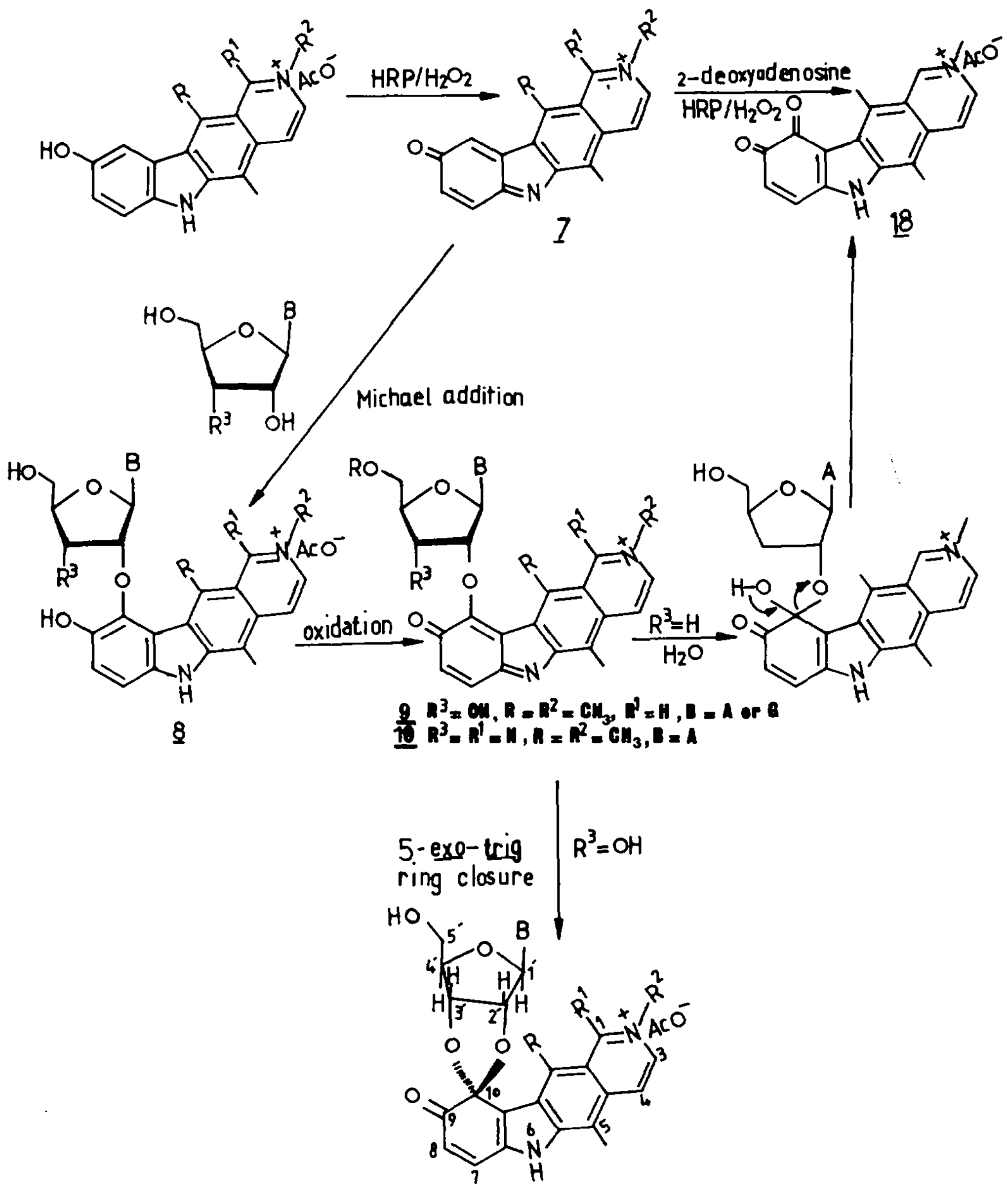
Abstract : Reaction of 9-hydroxypyrido[4,3-b]carbazole derivatives under oxidative conditions (biochemical : HRP/H_2O_2, chemical : Cu_2Cl_2/Pyridine/O_2) has been found to furnish regio- and stereo-selectively a ketalic linkage at the 10-position of this type of compound. Detailed structure elucidation of the products has been given. The regio- and stereo-specificity of the reaction has been demonstrated to be due to π-π stacking between the ribonucleosidic base and the 9-hydroxypyridocarbazole nucleus. Acid Catalysed isomerisation of 11 to 29 has also been studied in detail. In the light of these results, it is proposed that besides the "classical" intercalation into DNA, this type of compound can also react with RNA. This reactivity could explain, at least partially, the pharmacological (antitumour) activity of these compounds and should lead to a promising field of research in molecular biology.

The plant alkaloids ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole) 1 and its natural occurring isomer olivacine (1,5-dimethyl-6H-pyrido[4,3-b]carbazole) 2 were isolated from various plants of the *Aspidosperma*, *Ochrosia* and *Tabernaemontana* genera, of the family Apocynaceae.[1] These alkaloids exhibit high cytotoxic activity against murine *leukaemia* L 1210 and various other cancerous cells.[2-5] Hydroxylation at the 9-position of these alkaloids enhances their cytotoxic and antitumoural activity.[6,7] Structures 3 and 4 have also been identified as metabolites of ellipticine and olivacine respectively.[8-10] Quaternization of the pyridinium nitrogen of 3 gave the most active anticancer compound 9-hydroxy-N-2-methylellipticinium acetate (9-OH-NME$^+$, NSC 264137) 5[11] which has recently been used in the treatment of osteolytic breast cancer metastases.[12,13]



1 R = CH_3, $R^1 = R^2 = R^3$ = H
2 R^1 = CH_3, $R = R^2 = R^3$ = H
3 R = CH_3, $R^1 = R^2$ = H, R^3 = OH
4 R^1 = CH_3, $R = R^2$ = H, R^3 = OH
5 R^1 = H, $R = R^2$ = CH_3, R^3 = OH
6 R = H, $R^1 = R^2$ = CH_3, R^3 = OH

A considerable amount of attention has been focused on the elucidation of the mechanism of action of this type of compound *in vivo*. However, it still stands at a curious intersection of several scientific frontiers. This type of compound binds to double stranded DNA with an affinity coefficient of about 10^{-5} M^{-1}- 10^{-6} M^{-1},[14] inhibits the polymerisation of tubuline[15] and intercalates



11 $R^1 = H, R = R^2 = CH_3, B = A$
12 $R^1 = H, R = R^2 = CH_3, B = G$
13 $R^1 = H, R = R^2 = CH_3, B = C$
14 $R^1 = H, R = R^2 = CH_3, B = U$
15 $R = H, R^1 = R^2 = CH_3, B = A$
16 $R = H, R^1 = R^2 = CH_3, B = G$
17 $R^1 = CH_3, R = R^2 = H, B = A$

Scheme 1

between DNA base pairs.[16,17] DNA intercalation has long been related to the cytotoxic activity of these compounds. However, the presence of a hydroxyl group *para* to the indolic nitrogen suggests that this class of antitumoural agents upon oxidation *in vivo* could generate quinone-imine type structures 7.[18] The thus generated quinone-imine in turn could co-valently bind to biomolecules (such as nucleic acid and proteins). Quinone-imine 7 is also analogous to the quinone methide intermediates proposed by Moore in his bioreductive alkylation model for various anticancer agents.[19] Studies of the various nucleophiles reacting with 5 under oxidative conditions have supported this hypothesis.[20-22] This was further supported by the isolation of 9-OH-NME$^+$ and 9-OH-NMO$^+$-10-S-glutathione conjugate, as bile excretion metabolites in rats treated with 5 and 6 respectively.[23]

Recently, working on the interactions of 9-OH-NME$^+$ 5 with nucleic acids, we reacted 5 with adenosine under bioxidative conditions (HRP/H_2O_2) to afford the adenosine adduct 11[22] and thus were led to revise the structure 9, previously assigned by Meunier *et al.*[24,25]

The structure of the adducts, obtained by the reaction of nucleosides and 9-hydroxypyridocarbazoles under oxidative conditions could indeed be an important aid for understanding the interactions of nucleic acids with 9-hydroxypyridocarbazole derivatives (and possibly other structurally related substrates). Therefore, a detailed study has been carried out to evaluate the structure, stereochemistry of the reaction products and mechanism of their reaction with various nucleosides. For the first time, this study has been extended to 9-hydroxy-N-2-methylolivacinium acetate (9-OH-NMO$^+$) 6.

In this paper, we present the details of the oxidations of 9-OHE$^+$ 3, 9-OHO$^+$ 4, 9-OH-NME$^+$ 5 and 9-OH-NMO$^+$ 6 under various oxidative (chemical and biochemical) conditions in the presence of oxygen bearing nucleophiles (such as methanol, ethylene glycol and ribonucleosides), the regio- and stereoselectivity of the reaction and the comparative reactivity of pyrimido- and purino-ribonucleosides.

Reaction of 9-OH-NME$^+$ 5 (1 eq) with ribonucleosides (5 eq) in phosphate buffer (pH 7.02) upon biochemical oxidation (Horse-Radish Peroxydase (HRP/H_2O_2) furnished the corresponding adducts 11-14 in a moderate yield. However, the same reaction with 9-OH-NMO$^+$ 6, using 9-OH-NMO$^+$ (1 eq) with ribonucleosides (5 eq) failed to yield the desired adducts (except guanoside adduct 16). In order to obtain the olivacinium adducts 15-17, reaction of 9-OH-NMO$^+$ 6 and 9-OHO$^+$ 4 were studied at pH 5.8 and using a high concentration of ribonucleosides (10 eq). Treatment of the mixture of 9-OHO$^+$ 4 (1 eq) and adenosine (5 eq) in DMF with cuprous chloride and pyridine also affords the corresponding adduct 17 in 23% yield. (Scheme 1).

Reaction of 9-OH-NME$^+$ 5 was also studied with 2'-deoxyadenosine and 3'-deoxyadenosine. Oxidation of 9-OH-NME$^+$ 5 with 2'-deoxyadenosine gave exclusively orthoquinone 18. However, similar reaction with 3'-deoxyadenosine gave an unstable adduct 10 which readily hydrolysed to the orthoquinone 18 as described earlier.[25]

During the course of these studies, it was observed that reactions with purinoribonucleosides (adenosine/guanosine) were faster than with the pyrimidoribonucleosides (cytidine/uridine). In order to compare the reactivity of pyrimido- and purino-ribonucleosides, one-pot comparative reactions of 9-OH-NME$^+$ (1 eq), adenosine (5 eq) and cytidine (5 eq) or uridine (5 eq) under oxidative conditions were carried out. During these reactions, it was shown that 9-OH-NME$^+$ reacted selectively with adenosine to yield 11 and none of the cytidine (13) or uridine (14) adduct was detected.

The spectral characteristics of the adducts were in agreement with the assigned structures 11-17. The protons present in the adducts were readily assigned by extensive decoupling and NOE experiments. Thus for example in 11, ^1H NMR spectrum (CD_3CO_2D/D_2O) at 400 MHz showed two mutually isolated AX systems at δ 8.25, 8.10 (J = 6.7 Hz) and δ 6.21, 7.66 (J = 10 Hz). The remaining three aromatic protons appeared as singlets at δ 8.45, 8.65 and 9.84. The two doublets at δ 8.25 and δ 8.10 (J = 6.7 Hz) were assigned as H3 and H4 respectively using NOE and decoupling experiments. Similarly, a singlet present at δ 9.84 accounted for H1, since it showed a strong NOE effect both with the 11-CH_3 and the N-2-methyl group. The two doublets at δ 6.21 and 7.66 (J = 10 Hz) separated from each other by 1.45 ppm, are characteristic of an α,β-unsaturated ketonic system and were assigned as H8 and H7 respectively. The presence of an AX system for H8 and H7 indicates adduct formation at position-10 and excludes alkylation at other possible sites (b and c). Figure 1.

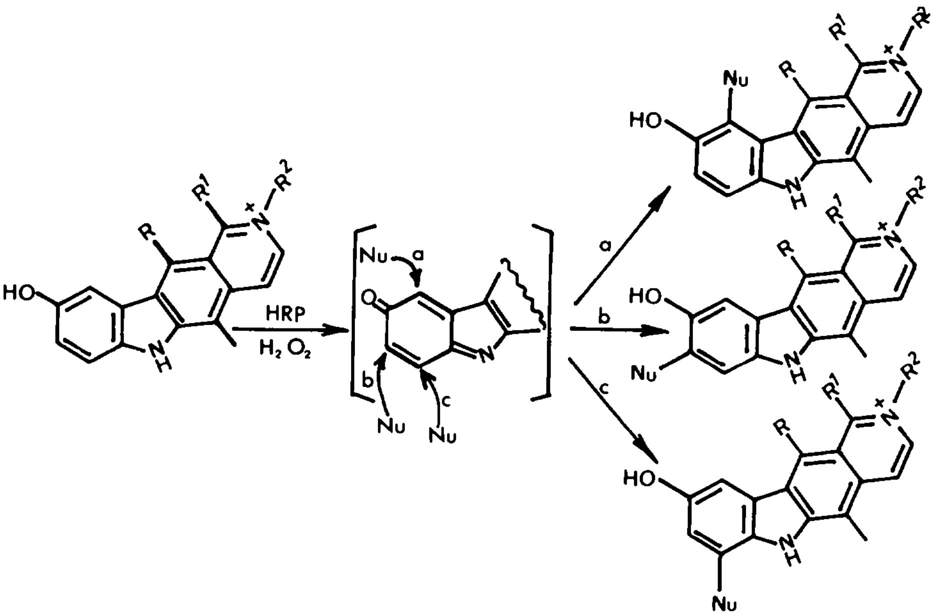


Figure 1

A downfield shift of the ribose ring protons in the adducts, when compared to the parent nucleoside and to alkylation by uridine (having no amino group) suggests alkylation by the ribose ring. The small coupling constants between H2' and H1' (J ≤ 1.0 Hz) and H3' and H4' (J ≤ 1.0 Hz) in all the ribonucleoside adducts indicate that both the 2' and 3'-OH groups are involved in the alkylation to give regio- and stereo-specifically a ketalic linkage at position-10 of these compounds.[26]

The M+2 peak was obtained for each ribonucleoside adduct cation in the FAB (Fast Atom Bombardment) mass spectrum. Mass fragmentation of the adducts has shown a common peak at 292 (in ellipticinium adducts) and 291 (in olivacinium adducts). Peaks at 291 and 292 could be accounted for as the orthoquinone (M^+ (cation) = 291) A and the semi-orthoquinone (M^+ (cation) = 292) B respectively. Formation of orthoquinone could be explained by the elimination of ribonucleoside as 2',3'-epoxy-ribonucleoside from the adducts. The appearance of the M+2 peak in the mass spectrum could be explained by the reduction of the generated quinone-imine in the ion source. Figure 2.

In view of the previously reported structure 9 for the adducts by Meunier et al.,[24,25] we decided to support our own structure by a ^{13}C NMR study of the adducts and of analogous model compounds. In the ^{13}C NMR C-10 should be quite

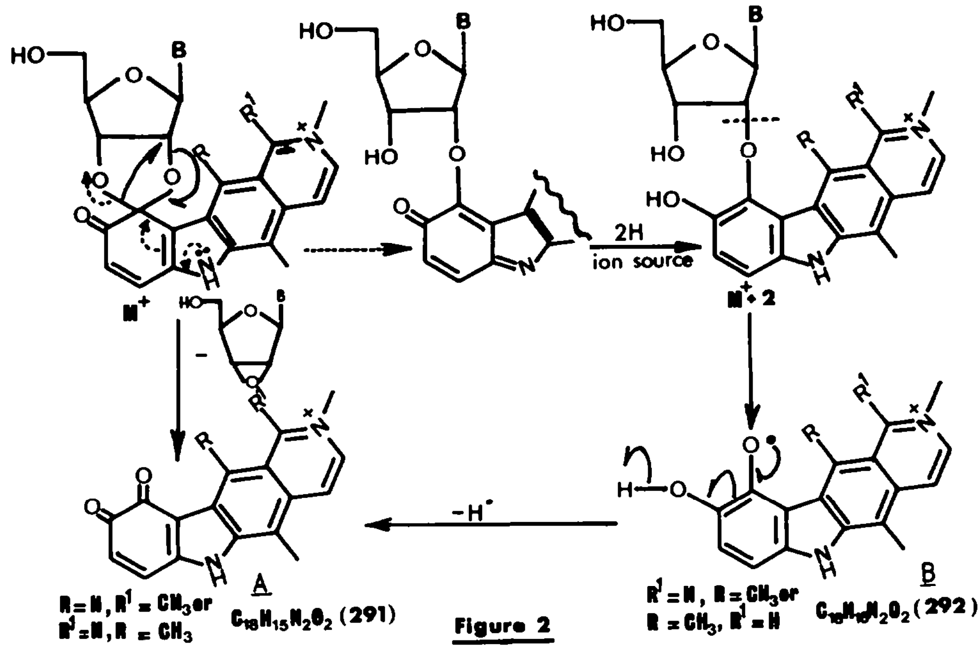
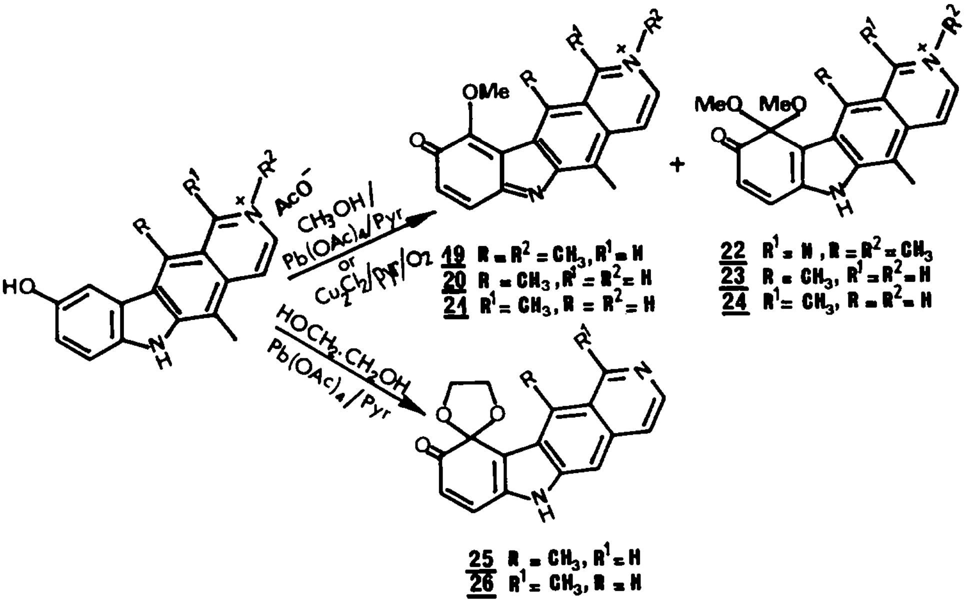


Figure 2

different in each of the two structures (enolic and ketalic). For obtaining model compounds 19, reaction of 9-OH-NME$^+$ 5 in methanol with cuprous chloride and pyridine was re-investigated.[20] Treatment of 9-OH-NME$^+$ 5 in anhydrous methanol with cuprous chloride (2 eq) in presence of pyridine afforded exclusively dimethoxy ketal 22 in 76% yield,[22] which was previously incorrectly reported as 10-monomethoxyquinone-imine ether 19 by Meunier et al.[20] For obtaining the monomethoxy adduct 20, we turned our attention to nonquaternised 9-hydroxyellipticine (9-OHE$^+$) 3. Oxidation of 9-OHE$^+$ 3 in anhydrous methanol with lead tetraacetate in presence of pyridine at room temperature furnished a mixture of monomethoxy ether 20 (16%) and dimethoxy ketal 23 (44%) in 3 hours. The mixture of 20 and 23 was separated on Sephadex LH-20 using CH_2Cl_2 as an eluent. Similarly, treatment of 9-hydroxyolivacinium acetate (9-OHO$^+$) 4 in anhydrous methanol with lead tetraacetate in presence of pyridine at room temperature for 3 hours yielded a mixture of 21 (14%) and 24 (62%) which were also separated on Sephadex LH-20 using CH_2Cl_2 as an eluent. Oxidation of 9-OHE$^+$ 3 and 9-OHO$^+$ 4 with lead tetraacetate in presence of pyridine and ethylene glycol were also investigated to yield 25 (44%) and 26 (44%) respectively. The spectral data of these compounds were compatible with the assigned structures.

The ^{13}C NMR spectra of 20, 23, 25 and 26 showed resonances for C-10 at δ 166.9, 94.50, 99.91, 100 and for C-9 (C:O) at δ 184.8, 197.35, 199.25, 199.5 respectively. The ^{13}C NMR data of these compounds are also in close agreement with the reported data for analogous systems.[27,28] Analysis of the ^{13}C NMR spectra of these compounds showed as expected that in the monomethoxy enolic structure 20 C-10 appeared about 66-72 ppm downfield while C-9 (C:O) showed 13-15 ppm upfield shift compared to the ketalic structure. The C-10 and C-9 (C:O) in the ^{13}C NMR spectra of the adducts, for example in 11 and 17, were observed at δ 105.19, 105 and δ 198.2, 199.4 respectively and therefore, further illustrate the formation of a ketalic linkage at position 10 of the 9-hydroxy-ellipticinium and-olivacinium adducts.



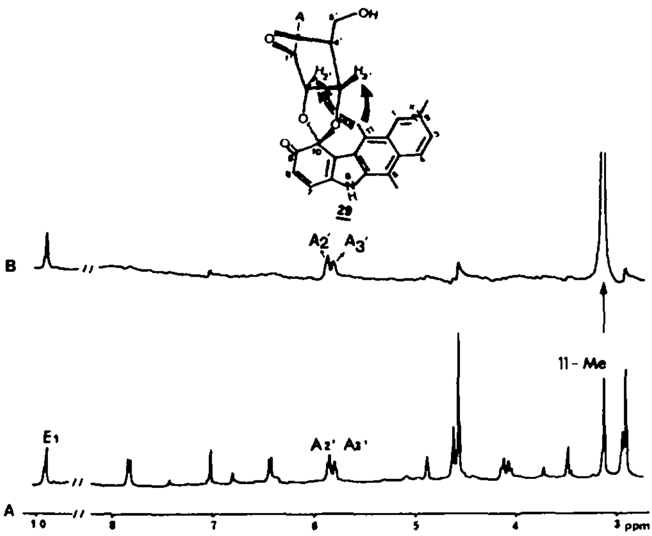
Scheme 2

The absolute configuration at C-10 of the 9-OH-NME$^+$ adduct was confirmed by two dimensional NMR studies.[22] In the case of each of the ellipticinium nucleoside adducts, the 11-CH$_3$ group showed a strong NOE with the H1' and H4' protons of the ribose ring. This confirmed the S-configuration at C-10 in 11-13.

Isomer 11 equilibrated to another isomer 29 (40-60%) along with a little orthoquinone (18) on standing in acetic acid in the presence of a drop of water after 15 days at room temperature. Treatment of 11 with glacial acetic acid at 67°C during 3 hrs gave the mixture of 11, 18, 29 and an unidentified product. On reaching the thermodynamic equilibrium 40-60% conversion of 11 to 29 was observed. The mixture of 11, 18 and 29 was separated on Sephadex LH-20 (4 x 30 cm^3) using MeOH as eluent. Spectral data of 18[29] and 29 were in close agreement with the assigned structure. Comparison of the ^{1}H NMR spectra (400 MHz, CD_3CO_2D/D_2O) of 11 and 29 indicate that the H2' and the 11-CH$_3$ group in 29 undergo upfield shifts of 0.27 and 0.21 ppm respectively. However, H1' showed a downfield shift of 0.21 ppm. The coupling constants between H1' and H2' ($J_{1',2'}$= 2.2 Hz) and H3' and H4' ($J_{3',4'}$=2.0 Hz) in 29 suggest that the ribose ring in this isomer adopts the O'-exo type conformation closely related to the O'-exo conformation in 11. Furthermore, the observed strong NOE effect between the 11-CH$_3$ group and H2' and H3' confirmed the R-configuration at C-10 in 29 (Figure 3).

The uridine adduct 14 was found to be a diastereomeric mixture of 10S and 10R as opposed to other ribonucleoside adducts 11-13 (10S). The chemical assignment of the two isomers in the mixture was made by comparing the ^{1}H NMR data with the 11 (10S) and 29 (10R). The major isomer in the mixture 14 was assigned to be 10S analogous to other ribonucleoside adducts.

In the case of the olivacinium adducts 15-17, the ribose ring also adopts an energetically unfavourable O'-exo conformation since small coupling constants between H1' and H2' ($J_{1',2'} \geq 1$) and H3' and H4' ($J_{3',4'} \geq 1$) were observed.[26]



A ^1H NMR spectrum (400 MHz, CD_3CO_2D + D_2O) of 29.
B NOE difference spectrum of 29 after irradiating at 11-CH_3.

Figure 3.

The absolute configuration of the olivacinium adducts at C-10 could not be confirmed by NOE experiments. However, the comparison of the chemical shifts of the protons of the ribose ring of the olivacinium adducts with the spectra of the S(11) and R(29) isomers of the ellipticinium adducts suggests that possibly the olivacinium adducts also have the S-configuration at C-10.

Mechanistically, adduct formation could be explained in the following manner : 9-hydroxy-ellipticinium or -olivacinium upon treatment with an oxidant (HRP/H_2O_2, Cu_2Cl_2/pyridine/O_2, $Pb(OAc)_4$/pyridine) give rise to an electrophilic quinone-imine of type (7). This quinone-imine 7 undergoes a regiospecific addition of the 2'-hydroxyl group of ribose. The resultant 10-(2'-O-ribonucleoside) alkoylated product (8) undergoes further oxidation followed by "5-*exo*-*trig*" ring closure *via* an intramolecular addition of the *cis* 3'-hydroxyl of the ribonucleoside to furnish regio- and stereo-selectively adducts 11-13 and 15-17. (Scheme 1).

No adduct formation was observed with 2'-deoxyadenosine, however, 3'-deoxyadenosine gave an unstable adduct 10 which has recently been characterised under reductive conditions.[30] It was therefore envisaged that the 2'-hydroxyl group of the ribose was involved in the first step of nucleophilic addition.

The regio- and stereo-selectivity of the reaction is attributed to the stacking interactions between the pyridocarbazole and the "base" part of the nucleoside. These stacking interactions bring the two reactive molecules into an appropriate proximity prior to the oxidation and Michael addition step.

In order to gain additional insight into this hypothesis, we initiated a detailed study of the interactions between 9-OH-NME$^+$ and adenosine. Following the protocol developed by Dimicoli and Helene[31] we monitored the various chemical shifts of one molecule in fixed concentration against the varying concentration of the other molecule in large excess. For analysis of the data, the following hypotheses were employed : a) Successive association constants are identical. b) Effects of magnetic anisotropy are additive. c) Only the magnetic anisotropy of the nearest neighbour is taken into account.

The experimental curves were fitted to the calculated ones, using a simple base algorithm (written on the Brucker 2,000 ASPECT).

Various autoassociation and affinity constants were calculated and are listed in Table 1.

Table 1.

Association Constant		Affinity Constant of Adenosine-9-OH-NME$^+$ a,b
Adenosine	9-OH-NME$^+$	
4.5 M^{-1}	1640 M	500 M^{-1}

(a) Large excess of 9-OH-NME$^+$ was used.

(b) When adenosine was used in excess, no conclusion could be drawn because of complexity of molecular associations.

The affinity constant, so obtained, is large enough to allow observation of intermolecular NOES between adenosine and the stacked ellipticinium protons. The observation of such effects indicates the spatial proximity of the related protons in the mixed aggregates.

As represented in Figure 4, a very strong NOE was observed between A2 and E7 protons, demonstrating their proximity in the aggregates.

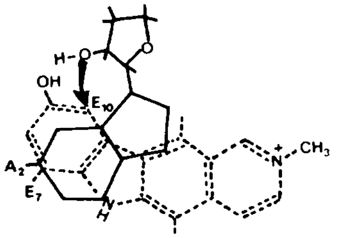
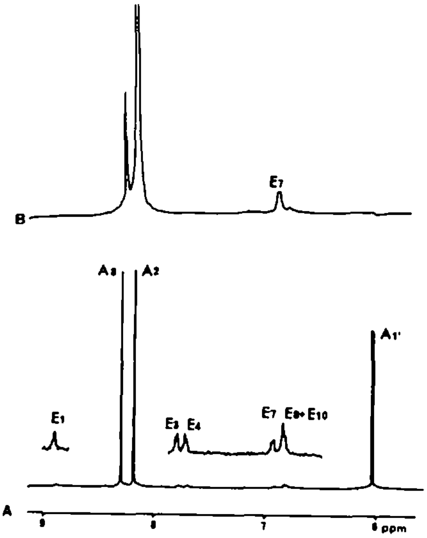


Figure 5



INTERACTION OF 9-OH-NME$^+$ WITH ADENOSINE

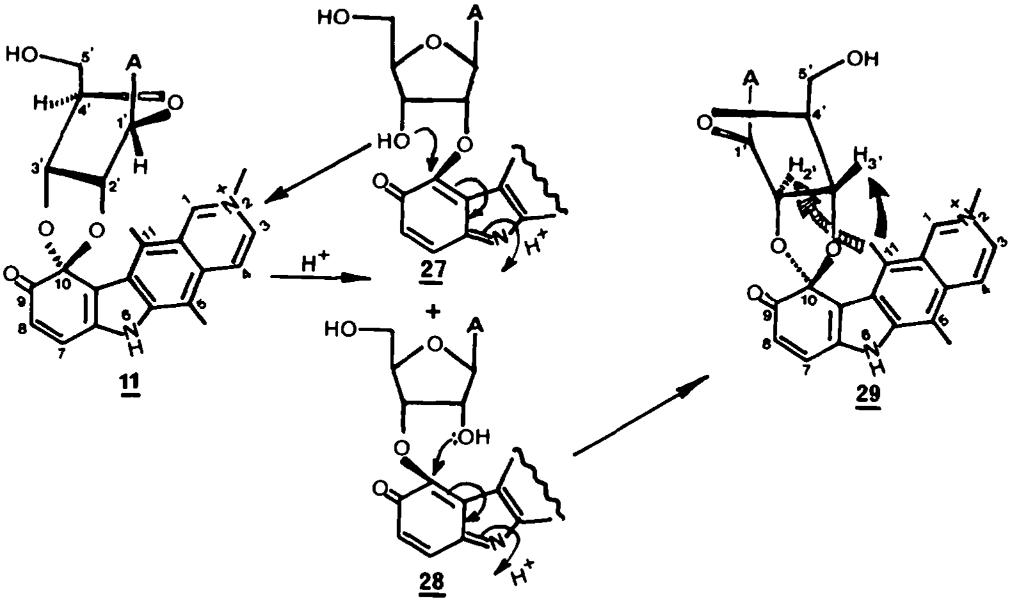
A ^{1}H NMR spectrum, A=adenosine=10M, 9-OH-NME$^+$=E=5.1^{-3}M

B Intermolecular relaxation A$_2$-E$_7$

Figure-4

The possible interaction geometry which incorporates this proximity and stacking between the ellipticinium and adenine ring is shown in Figure 5. This stacking brings the 2'-hydroxyl group of the ribose and the reactive C-10 centre to a favourable disposition (Fig. 5) for the first addition step and could explain the regioselectivity of the reaction. Such a very strong interaction, on the other hand, will considerably slow down the reaction of 2'-deoxyribonucleosides by maintaining a large distance between the C-10 centre and 3'-hydroxyl group, and consequently make the oxidation to the orthoquinone the predominant reaction pathway (Scheme 1). Finally, the stereoselective ring closure that follows the second oxidation step would be again governed by related stacking interactions.

Isomerisation of 11 to 29 under acidic conditions could be explained on the basis of a non regiospecific ring opening of a ketooxalone ring to furnish 10-(2'-O-adenosyl)-9-OXO-NME$^+$ 27 and 10-(3'-O-adenosyl)-9-OXO-NME$^+$ 28. 10-(3'-O-adenosyl)-9-OXO-NME$^+$ 28 on ring closure gave the isomer 29 while 10-(2'-O-adenosyl)-9-OXO-NME$^+$ 27 yielded back the starting material 11 (Scheme 3).



Scheme 3

In conclusion, this study has revealed the following facts :

(a) Reaction of 9-hydroxypyrido[4,3-b] carbazole with ribonucleosides under oxidative conditions is regio- and stereo-selective in nature. The remarkable regio- and stereo-selectivity of the reaction has been demonstrated to be due to stacking between the ribonucleosidic bases and the pyridocarbazole.

(b) Reaction of 9-hydroxypyridocarbazole derivatives with purinoribonucleosides is faster than with pyrimidoribonucleosides. The reaction of uridine with 9-OH-NME$^+$ is non stereoselective. These observations illustrate that stacking between purinoribonucleosides and 9-hydroxypyridocarbazole could be stronger than with pyrimidoribonucleosides.

(c) *Cis* 2'- and 3'-hydroxyl groups in the ribonucleosides are essential for the stable adduct formation. Nevertheless, co-valent bond formation with 3'-deoxyribonucleosides cannot be ruled out. However, our study with di- and tri-ribonucleotides (where 2'-free hydroxylgroups are present along with free *cis* 2' and 3'-OH groups) has indicated that reaction with *cis* 2' and 3'-hydroxyl groups is preferred.[32,33]

(d) Formation of a stable ketalic linkage at the 10 position of 9-hydroxypyrido [4,3-b] carbazole derivatives has demonstrated that besides the "classical" intercalation into DNA, alkylation of RNAs by this class of compounds could also be a possible explanation for their antitumoural activity. For example : this type of compound could alkylate at the terminal end of t-RNAs to stop the formation of aminoacyl t-RNAs ; they can also react at the level of the "cap" present at the 5'-end of mRNAs or poly-A tail of the m-RNAs and consequently could inhibit the biosynthesis of proteins.[22,32] Blockage of the synthesis of the polyribonucleotides and polypeptide chains at an elongation step by olivacine in *E. coli*[34] is compatible with this hypothesis.

Presently, the application of these reactions are under investigation to test the validity of this hypothesis and to design other potent antitumoural agents.

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer 297 infrared spectrophotometer. UV spectra were obtained on Perkin-Elmer Lambda 5 UV/VIS spectrophotometer. Mass spectra under electron impact conditions were recorded on a AEI MS-50 mass spectrometer at 70 e.v. ionizing voltage. Fast Atom Bombardment (FAB) mass spectra were measured with an AEI mass spectrometer modified for FAB mass spectrometry. ^1H NMR and ^{13}C NMR spectra were obtained on Brucker W.M 400 and SY-200 spectrometer. NMR spectra were measured in an appropriate solvent with TMS or the chemical shift of the deuterated solvent as standard. Chemical shifts are expressed in δ ppm downfield from TMS. Chemical shifts and coupling constants were obtained from first order analysis of the spectra. Assignments were confirmed by means of double irradiation and NOE experiments. Assignment of the carbons in ^{13}C NMR could be exchanged carbon having the same asterisk (*).

The selectivity & progress of the reactions were checked by High Performance Liquid Chromatography (HPLC) on a Waters Associates System, using a µ Bondapack C-18 reverse phase column and a mixture of methanol/1 x 10^{-3} M ammonium acetate 1:1 (vol/vol) as eluent, after acidification to pH 4.5 with acetic acid. A UV detector spectrometer, operating at 254 n.m. was used to detect ellipticine and olivacine derivatives. Chromatography, if necessary, was performed on Sephadex LH-20, which was swelled in an eluent for 3-4 hours prior to use as an adsorbent.

9-Hydroxyellipticine,[4] 9-hydroxyolivacine,[35] 9-hydroxy-N-2-methylolivacinium acetate[36] were synthesised following the literature procedures. 9-Hydroxy-N-2-methyl-ellipticinium acetate was generously donated by Sanofi (France). Horse Radish Peroxidase (HRP) and nucleosides were purchased from the Sigma Chemical Company.

Reaction of 9-OH-NME$^+$ (5) with ribonucleosides in presence of HRP/H_2O_2 :

H_2O_2 (1M, 250 µl) was added to a solution of 9-OH-NME$^+$ 5 (0.1 mmol), ribonucleoside (0.5 mmol) and HRP (0.2 mmol) in phosphate buffer (0.05 M, pH 7.02 40 ml), dropwise at room temperature. The reaction mixture was stirred at this temperature for 5 min.-1 hr (5 min. in the case of adenosine and guanosine and 1 hr in the case of cytidine and uridine adducts). The ribonucleoside adducts precipitated out. Sodium chloride solution (5%, 10 ml) was added for complete precipitation of the adducts. The precipitated adduct was filtered, washed with sodium chloride solution (5%), followed by distilled water (1 ml) and dried. The resultant adducts were solubilized in the mixture of methanol-acetic acid (1:1 Vol/Vol, 2 ml) and precipitated with diethyl ether (10 ml). The resulting suspension was centrifuged and the supernatant liquid was decanted. The solid so obtained was redissolved in methanol (2 ml) reprecipitated with diethyl ether (10 ml) centrifuged and decanted. The adducts so obtained were washed with diethyl ether (10 ml x 3) and dried to furnish 11 to 14 (62 to 85% yield).

11 : Yield = 82% ; UV-visible spectrum in H_2O : ε in M^{-1} cm^{-1}, 9049, 23737, 41132, 34493 at λ 365, 308, 273 and 210 nm respectively ; ^1H NMR (400 MHz, CD_3CO_2D + 1 drop of D_2O) δ 2.73(s, 3H, 5-CH_3), 3.30(s, 3H, 11-CH_3), 3.93 (m, 2H, 5'-CH_2), 4.57 (s, 3H, N-CH_3), 4.80 (m, 1H, $J_{3',4'} \leqslant 1$ Hz, $H_{4'}$), 5.77 (d, $J_{2',3'}$ = 7 Hz, $H_{3'}$), 6.10 (d, 1H, $J_{2',3'}$ = 7 Hz, $J_{1',2'} \leqslant 1$ Hz, $H_{2'}$), 6.67 (s, 1H, $H_{1'}$), 6.21 (d, 1H, $J_{7,8}$ = 10 Hz, H_8), 7.66 (d, 1H, $J_{8,7}$ = 10 Hz, H_7), 8.10 (d, 1H, J = 6.7 Hz, H_4), 8.25 (d, 1H, $J_{3,4}$ = 6.7 Hz, H_3), 8.45 (s, 1H, H_2A), 8.65 (s, 1H, H_8A), 9.84 (s, 1H, H_1) ; ^{13}C NMR (50 MHz, D_2O) δ 12.6 (CH_3), 17.6 (CH_3), 48.4 (N-CH_3), 62.3 (C-5'), 85.4 (C-3'), 86.3 (C-2'), 88.6 (C-4'), 89.6 (C-1'), 105.2 (C-10), 108.5 (C-5), 114.7 (C-11), 118.93 (C-5A)*, 121.8 (C-11)*, 122.4 (C-4), 127.8 (C-7), 129.7 (C-11a)**, 130.6 (C-10b)**, 131.5 (C-3), 131.9 (C-4a), 135.8 (C-8), 141 (C-8A), 141.5 (C-5a)***, 142 (C-5b)***, 148.5 (C-1), 148.8 (C-4A), 153.1 (C-2A), 155.5 (C-6A), 198.2 (C-9) ; MS(FAB, M^+ = 599, M^+ cation = 540) m/e 542 (M cation + 2)$^+$.

12 : Yield = 85% ; UV-visible spectrum in H_2O : ε in M^{-1} cm^{-1}, 4354, 9247, 27024 at λ369.8, 312 and 274 nm respectively ; ^1H NMR (400 MHz, CD_3CO_2D + 1 drop of D_2O) δ 2.82 (s, 3H, 5-CH_3), 3.25 (s, 3H, 11-CH_3), 3.90 (m, 2H, 5'-CH_2), 4.50 (s, 3H, N-CH_3), 4.75 (m, 1H, $J_{3',4'} \leqslant 1$ Hz, $H_{4'}$), 5.70 (d, 1H, $H_{3'}$), 6.0 (d, 1H, $J_{1',2'} \leqslant 1$ Hz, $H_{2'}$), 6.18 (d, 1H, J = 9.6 Hz, H_8), 6.37 (s, 1H, $H_{1'}$), 7.58 (d, 1H, J = 9.6 Hz, H_7), 8.10 (d, 1H, J = 6.6 Hz, H_4), 8.27 (d, 1H, J = 6.6 Hz, H_3), 9.70 (s, 1H, H_1); MS (FAB, M^+ = 615, M^+ cation = 556) m/e 558 (M cation + 2)$^+$.

13 : Yield = 70% ; UV-visible spectrum in H_2O : ε in M^{-1} cm^{-1}, 9689, 22461, 35601, 30438 at λ 360, 312, 276 and 212 nm ; ^1H NMR (400 MHz, CD_3CO_2D + 1 drop of D_2O) δ 2.70 (s, 3H, 5-CH_3), 3.11 (s, 3H, 11-CH_3), 3.88 (m, 2H, 5'-CH_2), 4.45 (s, 3H, N-CH_3), 4.74 (m, 1H, $J_{4',3'} \leqslant 1$ Hz, $H_{4'}$), 5.02 (d, 1H, $J_{2',3'}$ = 7.4 Hz, $J_{2',1'} \leqslant 1$ Hz, $H_{2'}$), 5.98 (d, 1H, J = 9.6 Hz, H_8), 6.13 (s, 1H, $H_{1'}$), 6.20 (d, 1H, J = 8.12Hz, H_5Cy), 7.53 (d, 1H, J = 9.6 Hz, H_7), 7.99 (d, 1H, J = 7.4 Hz, H_4), 8.12 (d, 1H, J = 8.12 Hz, H_6Cy), 9.67 (s, 1H, H_1) ;

MS (FAB, M^+ = 575, M^+ cation = 516) m/e 518 (M cation + 2)$^+$.

14 : Yield = 62% ; UV-visible spectrum in H_2O: ε in M^{-1} cm^{-1} 8343, 16941, 32612, 24067 at λ 370, 312, 275 and 214 nm ; ^1H NMR (400 MHz, CD_3CO_2D + 1 drop of D_2O) δ 2.81 (s, 3H, 5-CH_3, R + S), 3.08 (s, 3H, 11-CH_3, R), 3.33 (s, 3H, 11-CH_3, S), 3.95 (m, 2H, 5'-CH_2, R + S), 4.52 (s, 3H, N-CH_3, R), 4.55 (s, 3H, N-CH_3, S), 4.64 (m, 1H, $J_{3',4'} \leq 1$ Hz, $H_{4'}$, R), 4.81 (m, 1 Hz, $J_{3',4'} \leq 1$ Hz, $H_{4'}$, S), 5.47 (d, 1H, $J_{2',3'}$ = 7.4 Hz, $J_{3',4'} \leq 1$ Hz, $H_{3'}$, S), 5.49 (d, 1H, $J_{2',3'}$ = 7.4 Hz, $J_{3',4'}$ 1 Hz, $H_{3'}$, R), 5.69 (d, 1H, J = 7.4 Hz, $H_{2'}$, R), 5.83 (d, 1H, J = 7.4 Hz, $H_{2'}$, S), 5.93 (d, 1H, $J_{5,6}$ = 7.8 Hz, H_{5u}, R + S), 6.20 (s, 1H, $H_{1'}$, S), 6.25 (d, 1H, $J_{7,8}$ = 9.6 Hz, H_8, S), 6.34 (d, 1H, $J_{7,8}$ = 9.6 Hz, H_8, R), 6.48 (s, 1H, $H_{1'}$, R), 7.65 (d, 1H, H_7, S), 7.70 (d, 1H, H_7, R), 7.95 (d, 1H, H_5, R + S), 8.23 (d, 1H, $J_{3,4}$ = 7.4 Hz, H_4, R + S), 8.37 (d, 1H, H_3, R + S), 9.82 (s, 1H, H_1, R), 9.84 (s, 1H, H_1, S) ; MS (FAB, M^+ = 576, M^+ cation = 517) m/e 519 (M cation + 2)$^+$.

Reaction of 9-OH-NMO^+ (6) with purinoribonucleosides (adenosine and guanosine in presence of HRP/H_2O :

A solution of H_2O_2 (250 µl, 1M in 0.05 M phosphate buffer, pH 5.8) was added dropwise to a solution of 9-OH-NMO^+ 6 (0.1 mmol), ribonucleoside (1 mmol for adenosine and 0.5 mmol for guanosine), HRP (0.2 mmol) in phosphate buffer (0.05 M, pH 5.8 , 40 ml) at room temperature. The resulting dark yellow solution was stirred for 0.5 hr and filtered. Adducts 15 and 16 were precipitated by an addition of sodium chloride solution (10%, 15 ml), filtered, washed with sodium chloride solution (10%), followed by water (1 ml), dried. The resultant adducts were purified on Sephadex LH-20 using MeOH as an eluent to furnish the corresponding 9-OH-NMO^+ adducts. The adducts so obtained were dissolved in methanol-acetic acid (1:1 Vol/Vol, 2 ml), precipitated with diethyl ether (10 ml x 3) and dried to furnish the pure adducts 15 (45%) and 16 (50%).

15 : UV-visible spectrum in H_2O : ε in M^{-1} cm^{-1} 9050, 23738, 41133, 34494 at λ 365, 308, 273 and 209 nm ; ^1H NMR (400 MHz, CD_3CO_2D + D_2O) δ 2.92 (s, 3H, 5-CH_3), 3.50 (s, 3H, 11-CH_3), 3.93 (m, 2H, 5'-CH_2), 4.53 (s, 3H, N-CH_3), 5.06 (m, 1H, $J_{4',3'} \leq 1$ Hz, $H_{4'}$), 5.80 (d, 1H, $J_{2',3'}$ = 5.9 Hz, $H_{3'}$), 6.27 (d, 1H, $J_{1',2'} \leq$ 1 Hz, $J_{2',3'}$ = 5.9 Hz, $H_{2'}$), 6.24 (d, 1H, J = 9.6, H_8), 6.90 (s, 1H, $J_{1',2'} \leq$ 1Hz, $H_{1'}$), 7.77 (d, 1H, J = 9.6 Hz, H_7), 8.30 (m, 2H, H_3 and H_4), 8.74 (s, 1H), 8.94 (s, 1H), 8.99 (s, 1H) ; MS (FAB, M^+ = 599, M^+ cation = 540) m/e 542 (M cation + 2)$^+$.

16 : UV-visible spectrum in H_2O : ε in M^{-1} cm^{-1}, 6920, 23033, 32687 at λ 370, 307 and 275 nm ; ^1H NMR (400 MHz, CD_3CO_2D + 1 drop of D_2O) δ 2.91 (s, 3H, 5-CH_3), 3.0 (s, 3H, 11-CH_3), 3.95 (m, 2H, 5'-CH_2), 4.47 (s, 3H, N-CH_3), 5.04 (m, 1H, $J_{3',4'} \leq$ 1 Hz, $H_{4'}$), 5.79 (d, 1H, $J_{2',3'}$ = 7.3 Hz, $H_{3'}$), 6.17 (d, 1H, $J_{1',2'} \leq$ 1 Hz, $J_{2',3'}$ = 7.3 Hz, $H_{2'}$), 6.62 (s, 1H, $H_{1'}$), 6.33 (d, 1H, J = 9.6 Hz, H_8), 7.80 (d, 1H, J = 9.6 Hz, H_7), 8.25 (d, 1H, H_4), 8.40 (d, 1H, H_3), 8.82 (s, 1H), 8.96 (s, 1H) ; MS (FAB, M^+ = 615, M^+ cation = 556) m/e 558 (M cation + 2)$^+$.

Reaction of 9-OHO^+ (4) with adenosine in oxidative conditions :

A. Oxidation with HRP/H_2O_2 :

To a solution of 9-OHO^+ (15 mg, 0.047 mmol), adenosine (88 mg, 0.33 mmol), HRP (0.1 mmol) in phosphate buffer (pH 5.8, 20 ml), H_2O_2 (150 µl, 1M) was added at room temperature. The reaction mixture was stirred for a further period of 3 hrs. Progress of the reaction was monitored by HPLC. The separated solid was filtered, washed with H_2O and dried. The solid so obtained was dissolved in a mixture of CH_3OH-CH_2Cl_2 (1:9 ml) and purified on Sephadex LH-20 using methanol as eluent to give 17 (12 mg, 48.6%).

B. Oxidation with CuCl/Pyridine :

Cuprous chloride (21 mg, 0.212 mmol) was added to a mixture of 9-OHO^+ (16 mg, 0.05 mmol), adenosine (66 mg, 0.25 mmol), pyridine (0.5 ml) in DMF (10 ml). The reaction mixture was stirred for a further period of 15 hrs. The solvent was removed *in vacuo* at 20°C. The residue was dissolved in methanol and filtered through celite. Removal of the solvent yielded an impure solid which was purified on Sephadex LH-20 using MeOH as eluent to give 17 (6 mg, 23%) ; UV-visible spectrum in MeOH : ε in M^{-1} cm^{-1} 3882, 11941, 22823 at λ 360, 294 and 255 nm ; ^1H NMR (400 MHz, CD_3CO_2D + $CDCl_3$) δ 2.75 (s, 3H, 5-CH_3), 3.11 (s, 3H, 11-CH_3), 3.9 (m, 2H, 5'-CH_2), 5.06 (d, 1H, $J_{3',4'}$ = 1.5 Hz, $H_{4'}$), 5.82 (dd, 1H, $J_{2',3'}$ = 7 Hz, $H_{3'}$), 6.21 (dd, 1H, $J_{2',1'}$ = 1.5 Hz, $H_{2'}$), 6.70 (d, 1H, $J_{1',2'}$ = 1.5 Hz, $H_{1'}$), 6.09 (d, 1H, J = 9.3 Hz, H_8), 7.52 (d, 1H, J = 9.2 Hz, H_7), 7.69 (d, 1H, J = 6.4 Hz, H_4), 8.10 (d, 1H, J = 6.4 Hz, H_3), 8.55 (s, 1H, H_1), 8.37 (s, 1H, H_{2A}), 8.52 (s, 1H, H_{8A}) ; ^{13}C NMR (50 MHz, CD_3OD-$CDCl_3$), δ 12.3 (CH_3), 22.5 (CH_3), 63.1 (C-5'), 85.5 (C-3'), 87.5 (C-2'), 90.2 (C-4'), 92.2 (C-1'), 105 (C-10), 111.5 (C-5), 114.9 (C-4), 115.5 (C-11a), 126.6 (C-8), 127.8 (C-10b), 131.6 (C-4a), 135.4 (C-7), 137.7 (C-3), 139 (C-6a)*, 140.2 (C-5a)*, 141.3 (C-8A), 149.9 (C-4A), 153.6 (C-2A), 156.3 (C-6A), 160.6 (C-1) and 199.4 (C-9) ; MS (FAB, M^+ = 525, M^+ cation 526) m/e 528 (M cation + 2)$^+$.

Isomerisation of S-adenosine-9-OH-NME^+ adduct (11) to R-adenosine-9-OH-NME^+(29) in acetic acid :

A solution of 11 (60 mg, 0.1 mmol) in glacial acetic acid (1 ml) was heated at 65°C for 3.5 hrs. The reaction mixture was concentrated in vacuo. The resultant mixture of compounds was separated on Sephadex LH-20 using MeOH as eluent to give 18 (8 mg, 27%), 29 (7.5 mg, 12.5%) and starting material 11 (10 mg, 16.6%).

18 : Spectral data are compatible with the data reported in the literature.[29]

29 : UV-visible spectrum in H_2O : ε in M^{-1} cm^{-1} 9078, 15793, 35096 at λ373, 312 and 271 nm ; ^1H NMR (400 MHz, CD_3CO_2D-D_2O) δ 2.88 (s, 3H, 5-CH_3), 3.09 (s, 3H, 11-CH_3), 4.06 (m, 2H, 5'-CH_2), 4.53 (s, 3H, N-CH_3), 4.86 (m, 1H, $J_{3',4'}$ = 2.9 Hz, $H_{4'}$), 5.76 (dd, 1H, $J_{2',3'}$ = 8 Hz, $H_{3'}$), 5.83 (dd, 1H, $J_{2',3'}$ = 8 Hz, $J_{1',2'}$ = 2.2 Hz, $H_{2'}$), 6.41 (d, 1H, J = 9.6 Hz, H_8), 6.98 (s, 1H, $H_{1'}$), 7.81 (d, 1H, J = 9.6 Hz, H_7), 8.23 (d, 1H, J = 6.6 Hz, H_4), 8.41 (d, 1H, J = 6.6 Hz, H_3), 9.88 (s, 1H, H_1) ; MS (FAB, M^+ 599, M^+ cation = 540) m/e 542 (M cation + 2)$^+$.

Reaction of 9-OHE^+ (3) with $Pb(OAc)_4$ in presence of MeOH :

To a solution of 9-OHE^+ (32 mg, 0.1 mmol) in dry methanol (10 ml) and pyridine (40 mg, 0.5 mmol), $Pb(OAc)_4$ (88 mg, 0.21 mmol) was added portionwise at room temperature. Instantaneously, the colour of the reaction changed from yellow to pink and slowly to red. The reaction mixture was further stirred at room temperature for 4.5 hrs filtered through celite. The solvent was removed under pressure and the resulting crude residue was purified on Sephadex LH-20 using CH_2Cl_2 as eluent to afford 20 (4.5 mg, 16%) and 23 (14 mg, 44%).

20 : IR($CHCl_3$) 1635 cm^{-1} (C=O) ; ^1H NMR (200 MHz, $CDCl_3$) δ 2.79 (s, 3H, CH_3), 3.06 (s, 3H, CH_3), 4.21 (s, 3H, OCH_3), 6.44 (d, 1H, J = 9.9 Hz, H_8), 7.37 (d, 1H, J = 9.9 Hz, H_7), 7.74 (d, 1H, J = 5.5 Hz, H_4), 8.60 (d, 1H, J = 5.5 Hz, H_3), 9.43 (s, 1H, H_1) ; ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.45 (5-CH_3), 16.70 (11-CH_3), 61.80 (OCH_3), 118.3 (C-4), 127.9 (C-5)*, 128(C-11)*, 128.4 (C-10b)*, 133.3 (C-8), 133.8 (C-4a), 135.6 (C-3), 138.4 (C-10a), 145.5 (C-7), 149 (C-5a), 150.2 (C-1), 155 (C-6a)**, 166.9 (C-10)**, 184.8 (C-9) ; MS (EI) m/e 290 (M^+), 275 (M^+-15).

23 : IR($CHCl_3$) 1675 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$) δ 2.78 (s, 3H, CH_3), 3.29 (s, 9H, 2 x OCH_3 and CH_3), 6.25 (d, 1H, J = 10.3 Hz, H_8), 7.51 (d, 1H, J = 10 Hz, H_7), 7.81 (d, 1H, 5.9 Hz, H_4), 8.43 (d, 1H, J = 5.9 Hz, H_3), 9.34 (bs, 1H, NH), 9.73 (s, 1H, H_1) ; ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.10 (5-CH_3), 14.20 (11-CH_3), 49.70 (2 x OCH_3), 94.50 (C-10), 110 (C-5), 112.5 (C-10a), 115.05 (C-4), 122.4 (C-11), 125.1 (C-8), 125.7 (C-11b)*, 126.7 (C-10b)*, 134.5 (C-3), 137.2 (C-6a)**, 137.9 (C-5a)**, 138.5 (C-7), 197.35 (C-9) ; MS (EI) m/e 322 (M^+), 290 (M^+-CH_3OH), 277 (M^+-45), 275 (M^+-47).

Reaction of 9-OHO^+ (4) with $Pb(OAc)_4$ in presence of MeOH :

Reaction of 9-OHO^+ (4) with $Pb(OAc)_4$ was performed as described above to give 21 (14%) and 24 (62%) .

21 : IR($CHCl_3$) 1635 cm^{-1} (C=O) ; ^1H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 4.25 (s, 3H, CH_3), 6.11 (d, 1H, J = 9.9 Hz, H_8), 7.01 (d, 1H, J = 9.9 Hz, H_7), 7.38 (d, 1H, J = 5.5 Hz, H_3), 7.88 (s, 1H, H_{11}), 8.20 (d, 1H, J = 5.5 Hz, H_3) ; MS (EI) m/e 290 (M^+), 275 (M^+-15).

24 : IR($CHCl_3$) 1675 cm^{-1} (C=O) ; ^1H NMR (400 MHz, $CDCl_3$) δ 2.81 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 3.42 (s, 6H, 2 x OCH_3), 6.14 (d, 1H, J = 10.3 Hz, H_8), 7.42 (d, 1H, J = 10.3 Hz, H_7), 7.71 (d, 1H, J = 5.9 Hz, H_4), 8.27 (d, 1H, J = 5.9 Hz, H_3), 8.56 (s, 1H, H_{11}), 10.1 (s, 1H, NH) ; MS (EI) m/e 322 (M^+), 291 (M^+-31), 276 (M^+-46).

Oxidation of 9-OHE^+ (3) with $Pb(OAc)_4$ in presence of ethylene glycol :

To a mixture of 9-OHE^+ (78.6 mg, 0.24 mmol), ethylene glycol (1 ml) and molecular sieves (1.0 gm) in dry ethyl acetate (5 ml), $CHCl_3$ (10 ml) and pyridine (273 mg, 3 mmol), $Pb(OAc)_4$ (264 mg, 0.54 mmol) was added at room temperature with stirring under an atmosphere of nitrogen. The reaction mixture was stirred for a period of 14 hrs, diluted with ethanol (25 ml) and filtered through celite. The solvent was removed under reduced pressure. The resulting residue was dissolved in a mixture of CH_2Cl_2-MeOH (9:1, 100 ml), washed with a saturated solution of $NaHCO_3$ (20 ml x 3) and dried over anhydrous $MgSO_4$. The organic layer was concentrated under reduced pressure. The resulting crude product was purified on Sephadex LH-20 using CH_2Cl_2 as eluent to yield 9 (34 mg, 44%). IR(KBr) 1670 cm^{-1} (C=O) ; ^1H NMR (400 MHz, DMSO-d_6) δ 2.76 (s, 3H, CH_3), 2.97 (s, 3H, CH_3), 4.45 (bs, 4H, -CH_2.CH_2-), 6.14 (d, 1H, J = 9.6 Hz, H_8), 7.65 (d, 1H, J = 9.6 Hz, H_7), 7.85 (d, 1H, J = 5.9 Hz, H_4), 8.32 (d, 1H, J = 5.9 Hz, H_3), 9.59 (s, 1H, H_1), 11.69 (s, 1H, NH) ; ^{13}C NMR (50 MHz, DMSO-d_6) δ 11.60 (5-CH_3), 14.80 (11-CH_3), 64.04 (-O-$(CH_2)_2$-O), 99.9 (C-10), 107 (C-10a), 110.97 (C-5), 115.7 (C-4), 122.92 (C-11)*, 124.5 (C-8), 125.6 (C-11a)*, 126.5 (C-10b)*, 139.18 (C-7), 150;25 (C-1), 199.3 (C-9) ; MS (EI) m/e 322 (M^+ + 2), 276 (M^+-C_2H_4O), 236 (M^+-84).

Oxidation of 9-OHO^+ (4) with $Pb(OAc)_4$ in presence of ethylene glycol :

Oxidation of 9-OHO^+ (4) with $Pb(OAc)_4$ in presence of ethylene glycol and the purification of the resultant crude product was carried out as described above to give 26 (44%). IR(KBr) 3250 (NH) and 1665 cm^{-1} (C=O) ; ^1H NMR (400 MHz, $CDCl_3$-DMSO-d_6) δ 2.82 (s, 3H, CH_3), 3.02 (s, 3H, CH_3), 4.55 (bs, 4H, -O-$(CH_2)_2$-O-), 6.04 (d, 1H, J = 10.3 Hz, H_8), 7.51(d, 1H, J = 10.3 Hz, H_7), 7.69 (d, 1H, J = 5.9 Hz, H_4), 8.22 (d, 1H, J = 5.9 Hz, H_3), 8.26 (s, 1H, H_{11}), 11.25 (bs, 1H, NH) ; ^{13}C NMR (50 MHz, $CDCl_3$-DMSO-d_6) δ 12.58 (5-CH_3), 23.08 (1-CH_3), 65.66 (O-$(CH_2)_2$-O), 100 (C-10), 105 (C-10a), 113.86 (C-11), 114.2 (C-5), 115.02 (C-4), 124.81 (C-8), 130.67 (C-4a), 134.35 (C-3), 137.79 (C-6a), 138.48 (C-7), 139.21 (C-5a), 159.36 (C-1), 199.5 (C=O) ; MS(EI) m/e 322 (M^+ + 2), 276 (M^+ - C_2H_4O) and 236 (M^+ - 84).

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