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UNEXPECTED REGIOSPECIFIC ALKYLATION OF THE ANTITUMOR AGENT N^2-METHYL-9-HYDROXYELLIPTICINIUM ACETATE WITH N, O OR S DONORS

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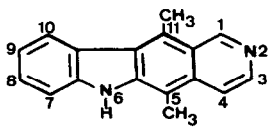
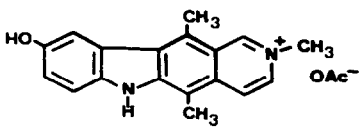
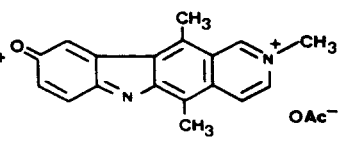
Summary : The quinone-imine derivative obtained by biochemical oxidation of 2, N^2-methyl-9-hydroxyellipticinium acetate, is found to react regiospecifically at C-10 with N, O ans S nucleophiles.

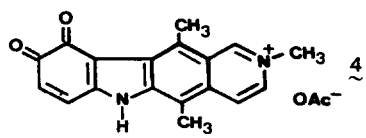
It has been reported[1] that some ellipticine derivatives manifest a much greater antitumour activity than ellipticine itself, 1 (5, 11-dimethyl-6H-pyrido[4,3b] carbazole). One of these derivatives, 2, N^2-methyl-9-hydroxyellipticinium, (9-OH-NME$^+$), which is obtained from 1 by hydroxylation at C-9 and methylation of the pyridine nitrogen, shows a very interesting activity and is currently under clinical investigation[2].

1 ELLIPTICINE 



2 9-OH-NME$^+$

3 9-OXO-NME$^+$ 

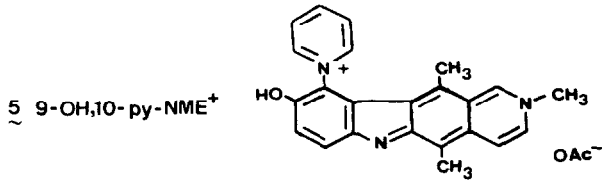


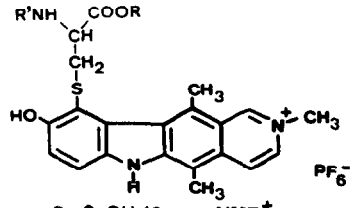
4 9,10-diOXO-NME$^+$

The respective positions of the hydroxyl and the indolic (N-H) groups, which are para to each other, actually suggest that the drug 2 might be activated *in vivo* through oxidative pathways. Our recent work[3] on the formation of two quinone derivatives, 3 and 4, via peroxidase oxidation of 2 by horseradish peroxidase (HRP) in presence of hydrogen peroxide, supports indeed this assumption.

Bearing in mind the alkylation of biological macromolecules by electrophilic quinone-imine derivatives and the implied consequences on biological activities[4a] , *we wish to report here our results about the addition of various nucleophiles (e.g. pyridine and cysteine derivatives) to 3,which occurs regiospecifically at C-10* ; we also want to report the formation of 7, the quinone-imine obtained by addition of a methoxy group at C-10 during the oxidation of 2 by molecular oxygen in presence of Cu^I compounds. *In both*

cases H-10 only is substituted by nucleophiles to give a new adduct though, for steric reasons, this preference for H-10 over H-8 is rather unexpected.



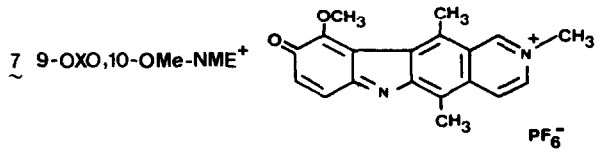


6 9-OH,10-cys-NME+

6a R=H R'=H

6b R=Me R'=H

6c R=H R'=Ac



- Pyridine-adduct 5

This adduct is prepared by oxidation of 2, using HRP/H_2O_2 in a phosphate buffer 0.05 M (pH 7.8) containing 5 % (v:v) of pyridine. After 30 min the solution is concentrated and purified on a Servachrom XAD-2 resin column [eluent : methanol-ammonium acetate 0.1 M, 5:95(v:v), followed by methanol]. The yield is 38 %. No other adduct can be detected . Structure 5 agrees with the ^1H NMR and mass spectroscopy data of the compound obtained. ^1H NMR (90 MHz) of 5 in D_2O : CD_3COCD_3, 50:50(v:v). 1.92 (s, 3p, CH_3COO^-), 2.16 (s, 3p, Me_5), 2.85 (s, 3p, Me_{11}), 4.50 (s, 3p, N -Me), 7.53 (d, 1p, J = 8.5 Hz, $H_{7(8)}$), 7.85 (d, 1p, J = 8.5 Hz, $H_{8(7)}$), 8.41 (m, 2p, H_3, H_4), 8.57 (m, 2p, H'_3, H'_5 pyridine) ; 8.96 (m, 1p, H'_4 pyridine), 9.19 (m, 2p, H'_2, H'_6 pyridine) and 9.67 (s, 1p, H_1). The signals at 8.57, 8.96 and 9.1 could not be observed in the ^1H NMR spectrum of the pyridine-d_5 adduct. M = 413 (cation = 354). Observed peak in EI : 353 (358 for pyridine-d_5 adduct).

- Cysteine-adduct 6

To avoid a direct contact between the oxidant mixture and the -SH containing molecules, the coupling reaction is performed after 3 has been extracted with an organic solvent (methylene chloride) in presence of ammonium hexafluorophosphate. Cysteine derivatives are dissolved in methanol before the addition to 3 in CH_2Cl_2 is carried out. As cysteine adducts are rather instable when dissolved, the solutions were always kept at low temperature (-20 °C). The products are isolated after concentration and precipitation in a mixture acetone-ether-hexane. The yield is 30-40 %.

The ^1H NMR and mass spectroscopy data of the compounds obtained are in agreement with structures 6a, 6b and 6c, which all present a covalent C_{10}-S bond. The mass and ^1H NMR data of 6c are given here, as an example. Mass data (M = 583, cation = 438) ; FD : molecular peak at 439 (MH^+) ; DCI (NH_3) : fragmentation peaks at 323 (ellip-S-CH_2), 309 (ellip-S) and 277.

^1H NMR (90 MHz) of 6c in D_2O : CD_3COCD_3 (3:1, v:v) : 1.63 (s, 3p, N-acetyl), 2.66 (s, 3p, Me_5)

3.19 (m, 2p -CH_2-cyst.), 3.37 (s, 3p, Me_{11}), 4.09 (m, 1p, -CH-cyst.), 4.51 (s, 3p, N^+-Me) 7.25 (d, 1p, H_7 or H_8), 7.47 (d, 1p, J = 9.8 Hz, H_7 or H_8), 8.20 (broad s, 2p, H_3 and H_4) and 9.66 (s, 1p, H_1).

Other S-adducts have also been prepared, acccording to the same procedure.

- Methoxy-quinone-imine 7

Hydroxylated molecules are usually readily oxidized by molecular oxygen in presence of Cu^I complexes [5,6]. The oxidation of 2, performed in methanol (2 is only poorly soluble in non-hydroxylated solvents) using molecular oxygen in presence of [(bipy)$_2$ Cu]Cl or [(bipy)$_2$ Cu] PF_6 is slow : The complete disappearance of 2 is achieved after 24 hours only, while the same reaction performed with CuCl in methanol in presence of a small amount of pyridine leads to 7 within one hour. After addition of NH_4PF_6 the solution is filtered twice on a Sephadex LH20 column [eluent : methanol]. After concentration of the solution, the product is precipitated by addition of ether. The yield is 75 %. The structure 7, a quinone-imine with a methoxy group at C-10, is compatible with IR, mass and ^{1}H NMR spectroscopy data. IR (CsBr) : 1670 cm^{-1} (C=O) and 1640 (C=N). Mass data (M=450, cation=305) ; EI : 306 (MH^+) ; CI (NH_3) : 307 (MH_2^+).
^{1}H NMR in CD_3COCD_3 : D_2O (2:1, v:v) : 2.96 (s, 3p, Me_5), 3.24 (s, 3p, OMe), 3.34 (s, 3p, Me_{11}), 4.60 (s, 3p, N^+-Me), 6.48 (d, 1p, J = 10,0 Hz, H_8), 7.91 (d, 1p, J = 10.0 Hz, H_7), 8.47 (2 d, 2p, J = 8.0 Hz, H_3, H_4) and 10.00 (s, 1p, H_1).

It is probable that the reaction proceeds through a nucleophilic attack of the methoxy group by a methoxy ligand of the copper complex, as described by Rogic et al. [6a,6b] for the oxidation of diphenols by O_2/Cu^I in methanol.

The striking feature of the adduct derivatives described here is that they are all obtained from 2 by a nucleophilic substitution at C-10. If the lack of reactivity of C-7 may be explained by the positive charge on N-2, which should favour the electrophilicity of positions 8 and 10 in 3, the much lower reactivity of position 8 compared to 10 is more difficult to anticipate due to the sterical hindrance introduced at C-10 by the proximity of a methyl group at C-11. If 3 were indeed formed as a metabolite, the N-acetyl-cysteine adduct 6 should be found *in vivo* as the conjugate derivated from the corresponding glutathion adduct. All these products have been tested *in vitro* on L1210 cells (Table 1) and have been found to be less cytotoxic than the starting compound 2.

Table 1. Cytotoxicity of the adducts 5, 6 and 7 (L1210 cells *in vitro*)

Compound	ID_{50} (48h)
9-OH-NME^+ 2	0.075 µM
pyridine add. 5	0.68 "
cysteine ester add. 6b	0.79 "
cysteine acetyl add. 6c	1.12 "
methoxy-10 cpd. 7	3.0 "

ID_{50} = concentration which inhibits the cell growth by 50 % in 48 hours

The toxicity of compounds such as 2 might thus be related directly to their oxidability and therefore to their alkylation by biological nucleophiles following their oxidation *in vivo* [i.e. a "bioxidative alkylation" as opposed to the "bioreductive alkylation" proposed by Moore[4b]]. Then the observed toxicity, which decreases from compound 5 to 7 can be explained as resulting from a nucleophilic addition on an oxidized form of 2.

Acknowledgements

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