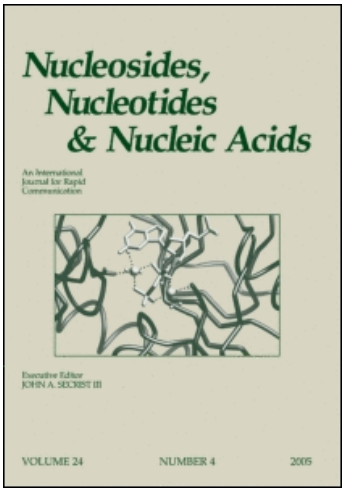

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RIBONUCLEOSIDE DIPHOSPHATE ADDUCTS WITH ELLIPTINIUM ACETATE, AN ANTITUMOR AGENT

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SUMMARY : The oxidized form of the antitumor agent elliptinium acetate is able to arylate adenosine and uridine 5'- diphosphate by attack of the 2'-O position of the ribose and cyclisation, leading to spiro derivatives. Ring opening occurs under reducing conditions and leads to the formation of adducts at 2' or 3' positions. Spiro and uncyclised adducts showed low cytotoxicity against L1210 cells in vitro.

Recently we have shown that the quinone imine form of elliptinium acetate (an antitumor agent, NSC 264137, NMHE) arylates purine nucleosides and nucleotides, by a regioselective attack of the 2'-O position of the ribose (1); a secondary cyclisation leads to a spiro derivative including both oxygen atoms 2' and 3' ; cyclisation products obtained with ribonucleosides can be cleaved under reducing conditions leading to the formation of adducts at position 2' or 3' (2,3).

Ribonucleotide reductase catalyses the rate-limiting step in the de novo synthesis of deoxyribonucleotides which are required for DNA replication (4). Two distinct classes of reductases have been described : one class is represented by the enzyme isolated from Escherichia coli, the other by the enzyme from Lactobacillus leichmannii. The latter uses adenosylcobalamin as a dissociable cofactor, whereas the E. coli enzyme lacks this requirement. Substrates of the first class (E. coli) are nucleoside 5'-diphosphates, those of the second are nucleoside 5'-triphosphates. Ribonucleotide reductases from mammalian sources are similar to those of the E. coli enzyme (4).

It was shown that antitumor property of several compounds, (pyrazoloimidazole, deoxyadenosine, hydroxyurea) are related to their

ability to inhibit ribonucleotide reductase (5). In the other hand synthetic nucleotides carrying modifications at C'2 or C'3 form a series of inhibitors (6).

Our purpose was to prepare diphosphate nucleosides substituted at 2' and 3' position by elliptinium acetate as potential ribonucleotide reductase inhibitors.

EXPERIMENTAL SECTION

Materials

NMHE was a gift of Sanofi (France). Horseradish peroxidase (HRP type VI, EC 1.11.1.7), alkaline phosphatase (from Escherichia coli, type III, EC 3.1.3.1) and nucleotides were obtained from Sigma Chemical Co (St Louis). Hydrogen peroxide solution (30 %), ammonium acetate and L-ascorbic acid were obtained from Merck. Usual laboratory reagents were purchased from Prolabo (Paris).

Spectrometric methods

NMR spectra were recorded on a Bruker (250 MHz) spectrometer operated in the Fourier transform mode (FT). The chemical shifts are expressed in ppm (δ) with tetramethylsilane (TMS) as internal standard.

A MM-ZAB was used for fast atomic bombardement (FAB) mass spectra (glycerol matrix).

Chromatographic methods

All HPLC studies were performed on a Waters chromatograph using a microbondapak C_{18} column and a mixture of methanol/10^{-2}M ammonium acetate (50/50, v/v) acidified to pH 4.5 with acetic acid as eluent. The detection of ellipticine derivatives was monitored by a UV spectrometer at 254 or 313 nm. RRT (HPLC relative retention time of adducts compared to NMHE) were calculated.

Preparation of the spiro ADP (IIa) and UDP (IIb) elliptinium adducts

0.2 ml of 0.1 M H_2O_2 was added dropwise to 242 mg (0.5 mmol) of UDP sodium salt, 3.4 mg of elliptinium acetate (0.01 mmol) dissolved in 5 ml of 1/15 M phosphate buffer pH8, in presence of 50 µl of 0.1 mM HRP. The

mixture was kept overnight in the refrigerator, the precipitate containing mainly side products was eliminated. The adduct was precipitated from the supernatant by addition of 2 ml of a 0.5 M NH_4PF_6 solution. The crude precipitate was washed twice with 0.5 M NH_4PF_6 once with methanol/ether 50/50 and once with ether and then dried under reduced pressure. 3.45 mg of the spiro UDP-elliptinium adduct IIb (hexafluorophosphate salt) was obtained (brown powder, dec. above 230°C, yield 42 % based on the initial NMHE) ; UV-visible (CH_3COOH, H_2O, 35/65) λ 445 nm (ε 2800 $M^{-1}cm^{-1}$), 365 (9900), 273 (44400), 225 (11400).

The spiro ADP-elliptinium adduct IIa (hexafluorophosphate salt) was obtained in 60 % yield in the same experimental conditions (brown-red powder, dec. above 230°C) ; UV-visible (CH_3COOH, H_2O, 35/65) λ 470 nm (ε 1900 M^{-1} cm^{-1}), 365 (8600), 272 (39000), 228 (9200).

Preparation of the ADP (IIIa or IVa) and UDP (IIIb or IVb) elliptinium ring-opened adduct

In the same experimental conditions as above, 30 min (at room temperature) after H_2O_2 addition, 0.4 ml of 0.1 M ascorbic acid (0.04 mmol) were added ; then the mixture was heated at 60° for 10 min and allowed to cool. The solution was applied to a C_{18} Sep-Pak cartridge. UDP excess was immediately eluted. Elution with H_2O gave 4.07 mg of UDP elliptinium adduct IIIb(or IVb) (sodium salt, orange powder, dec. above 230°C), corresponding to 41 % yield based on the initial NMHE ; UV-visible (H_2O) λ 442 nm (ε 2000 M^{-1} cm^{-1}), 362 (2700), 255 (18300). ADP elliptinium adduct IIIa(or IVa) (sodium salt, orange-red powder, dec. above 230°C) was obtained in 40 % yield in the same experimental conditions ; UV-visible (H_2O) λ 450 nm (ε 2800 $M^{-1}cm^{-1}$), 373 (3800), 303 (15600), 254 (23800), 218 (17500).

Phosphatase hydrolysis of the spiro ADP and UDP elliptinium adducts

15 units of alkaline phosphatase were added to 1 µmol of ADP (or UDP) elliptinium adducts IIa (or IIb) in 1 ml 0.1 M Tris buffer at pH 8.5 and incubated at 37°C. A 0.2 ml sample was then added to 1 ml of a solution of 10 % ascorbic acid/ammonium molybdate/H_2O (1/6/3), the mixture was warmed up to 45°C for 20 min and the color development was read at 820 nm, the color obtained corresponded to 1.8 µmol H_3PO_4.

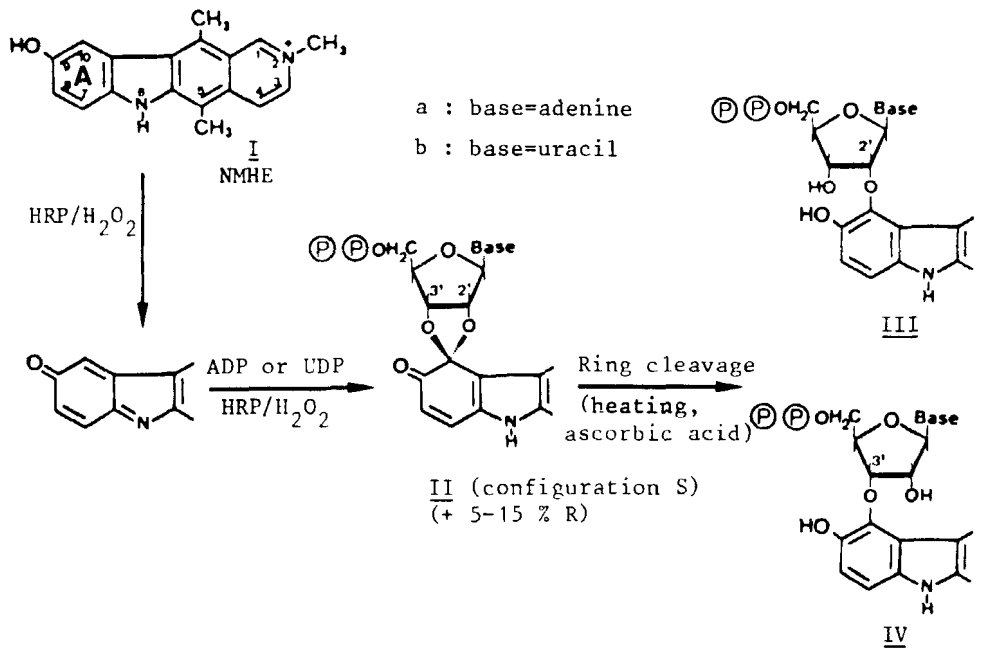
Cytotoxicity experiments

Inhibition of cell growth was determined with L1210 leukemia cells in vitro as previously described (8). The inhibitory efficiency of the elliptinium adducts is expressed in terms of drug concentration that reduces the number of cells by x % as compared to control after 48 h.

RESULTS and DISCUSSION

Structure of spiro nucleotides elliptinium adducts

Oxidation of NMHE I by the horseradish peroxidase/hydrogen peroxide (HRP/H_2O_2) system leads to the quinone-imine NMOE which is an highly electrophilic alkylating intermediate : it can react with an excess of H_2O_2 (formation of 9,10-dioxo-NMHE, a side product of the peroxidase oxidation), or alkylate nucleotides on the sugar moiety giving the adducts II, where the carbon 10 of elliptinium skeleton is linked to both 2' and 3' oxygen atoms of the sugar moiety (1,2,7) (for the detailed mechanism of the formation of II see reference 3) (Scheme I).



SCHEME I. Reactivity of oxidized form of NMHE with nucleoside diphosphates.

^1H NMR spectra of spiro ADP and UDP elliptinium adducts are similar to those of other known spiro adducts (7) (see table I) : (i) on the elliptinium moiety, the absence of a resonance corresponding to H_{10} and two widely separated H_7 and H_8 resonances are interpreted as C_{10} being the fixation site and a conjugated ketone structure from C_7 to O_9 (1,2,7) (ii) on the nucleotide part, the significant modifications of ribose proton resonances (low values for the coupling constants $J_{H1'-H2'}$ and $J_{H3'-H4'}$) indicate that the ribose stays in the energetically unfavourable O'-exo conformation (2).

As previously observed with AMP and UMP adducts, HPLC profile of the spiro UDP elliptinium adduct II revealed another peak ; by comparison with the analogous UMP adducts (7), we tentatively attribute to the major component the configuration S and to the minor component (15 %) the configuration R. The absolute configuration at carbon 10 of the minor isomer has been established by NOE in the case of AMP elliptinium adduct (7).The minor spiro ADP-elliptinium adduct was negligible under the conditions used.

The evidence of the existence of two phosphate groups was established by enzymatic hydrolysis : H_3PO_4 liberated after treatment of ADP or UDP elliptinium adduct by alkaline phosphatase was twice higher than H_3PO_4 obtained with AMP or UMP adduct under the same conditions.

Structure of ADP (IIIa,IVa) and UDP (IIIb,IVb) elliptinium ring-opened adducts

We have shown that ring cleavage of the spiro derivative can be performed by heating (50-60°C) an aqueous solution of this compound in the presence of ascorbic acid (3). It leads to the formation of adducts at position 2' or 3' (III or IV).

The adducts IIIa IIIb (or IVa, IVb) were identified on the basis of ^1H NMR. The AB type pattern observed for H_7 and H_8 is in agreement with the ring A being aromatic, and the value of 7-8 Hz of $J_{H1'-H2'}$ which is quite different from the small value observed for II (<1 Hz), indicates that the sugar ring is not blocked in an O'-exo configuration.

Under the experimental conditions used (4 equivalents of ascorbic acid) only one ADP-NMHE adduct was observed ; on the contrary a mixture of two UDP-NMHE adducts (IIIb + IVb) was obtained in the ratio of 80/20. In the absence of X-Ray structure determinations for III and IV, we

TABLE 1. ^1H NMR data for adducts IIa, IIb and IIIa, IIIb, IVb

	Ellipticinium								Ribose					Base	
	H_1	H_3	H_4	H_7	H_8	2-Me	5-Me	11-Me	H'_1	H'_2	H'_3	H'_4	H'_5 H''_5	H_2/H_5	H_8/H_6
Spiro ADP-adduct IIa (S)	9.88 s	8.27 d J~8Hz	8.47 d J~8Hz	7.75 d J~8Hz	6.38 d J~8Hz	4.55 s	2.92 s	3.44 s	6.75 s	6.26 b.s.	5.85 b.s.	5.12 b.s.	4.25 b.s.	8.55 s	8.7 s
ADP adduct IIIa	9.87 s	8.22 d J 7.68Hz	8.29 d J 7.68Hz	7.19 d J 8.40Hz	7.05 d J 8.40Hz	4.56 s	2.76 s	3.53 s	6.28 d J 8.04Hz	5.68 q	5.10 d J 4.75Hz	4.48 b.s.	4.36 4.17 b.s.	7.79 s	7.8 s
Spiro UDP adduct IIb (S)	9.88 s	8.20 d J 6.85Hz	8.37 d J 6.85Hz	7.93 d J 8.22Hz	5.92 d J 8.22Hz	4.53 s	2.85 s	3.29 s	6.20 s	5.82 b.s.	5.51 b.s.	4.96 b.s.	4.25 b.s.	6.30 d J 10Hz	7.64 d J 10Hz
(isomer R)			(8.51)				(3.83)	(3.06)	(6.50)				(4.37)	(6.64)	(7.77)
UDP adduct IIIb	9.93 s	8.18 d J 7.68Hz	8.28 d J 7.68Hz	7.27 d J~8Hz	7.23 d J~8Hz	4.52 s	2.79 s	3.54 s	6.21 d J 7.7Hz	5.07 m	4.93 m	4.29 b.s.	4.05 b.s.	4.79 d J 7.5Hz	7.02 d J 7.5Hz
(isomer IVb)	(9.82)						(4.50)	(2.82)	(3.62)	(6.34)					

s singlet ; d doublet ; b.s. broad signal ; q = quintuplet ; m multiplet. Solvent used : CD_3-COOD + D_2O (50/50).

TABLE 2. HPLC characteristics and quantitative evaluation of adduct formation between elliptinium and nucleoside diphosphates.

	HPLC RRT[(a)]	Yield[(b)]
Spiro ADP-NMHE adduct IIa	0.99[(c)]	60 %
ADP-NMHE adduct IIIa or IVa	1.39[(d)]	40 %
Spiro UDP-NMHE adduct IIb	0.62	42 %
UDP-NMHE adduct IIIb or IVb	1.57[(d)]	41 %

(a) Relative retention time on HPLC compared to the RT of NMHE under standard conditions (see experimental section) ; (b) yield (%) calculated with respect to initial NMHE in conditions of preparative experiments ; (c) a well-defined separation between IIa and NMHE is obtained when the eluant is acidified to pH3 with CF_3 COOH : RRT is then equal to 0.52. (d) broad peaks.

cannot exclude absolutely the possibility that the spectroscopic data for the compound which we consider to be III might be in fact for IV.

FAB mass spectra of ADP-NMHE adduct showed the molecular ion M^+ at 702, and peaks at 687 (M^+-CH_3), 622 (M^+-HPO_3). UDP-NMHE adduct showed peaks at 679 (M^+), 599 (M^+-HPO_3), 518 (M^+-(HPO_3 + H_2PO_3)).

Cytotoxicity studies on nucleotides-elliptinium adducts

Murine leukemia L1210 cells cultured in vitro were used for cytotoxicity studies. Cytotoxicity of spiro UDP and ADP-NMHE adducts was very low : 1.5 µg/ml spiro ADP adduct IIa (2.1 µM) led to 7 % inhibition, 4 µg/ml spiro UDP adduct IIb (5.9 µM) to 34 % inhibition.

In addition ring-opened UDP and ADP adducts exhibited negligeable cytotoxicity : 10 µg/ml ADP adduct IIIa (13 µM) gave only 11 % inhibition, 10 µg/ml UDP adduct IIIb (13.5 µM) led to 13 % inhibition.

In conclusion, the antitumor elliptinium acetate NHME is able after oxidation to behave as a strong electrophilic agent for the sugar

part of nucleotides giving two spiro derivatives (IIa, IIb) the cleavage of them under reducing conditions leads to the formation of adducts at position 2' or 3'. The adducts formed with ADP and UDP do not have the expected high cytotoxicity. However, we cannot discard the possibility of a ribonucleotide reductase inhibitor activity of those adducts, as several phenomenons might occur inside the cells, leading to a negative cytotoxic effect. Further studies on purified ribonucleotide reductases are required to know if these ribonucleotide elliptinium adducts might be potential inhibitors of this enzyme which is an important target in the design of new antiviral or antitumoral agents.

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REFERENCES

1- J. Bernadou, B. Meunier, G. Meunier, C. Auclair and C. Paoletti, Proc. Nat. Acad. Sci. USA, 81, 1297, (1984).

2- V.K. Kansal, S. Funakoshi, P. Mangeney, P. Potier, B. Gillet, E. Guittet and J.Y. Lallemand, Tetrahedron letters, 25, 2351-2354, (1984)

3- G. Pratviel, J. Bernadou and B. Meunier, J. Chem. Soc. Chem. Commun. 60-62, (1985).

4- L. Thelander and P. Reichard, Ann. Rev. Biochem. 48, 143-158, (1979).

5- A. Sato, G.L. Carter, P.E. Bacon and J.G. Cory, Cancer Res. 42, 4353-4357, (1982).

6- M. Lammers and H. Follmann, Structure and bonding, Springer-Verlag, Berlin, 54, 27, (1983).

7- G. Pratviel, J. Bernadou, T.B.T. Ha, G. Meunier, S. Cros, B. Meunier, B. Gillet and E. Guittet, J. Med. Chem., 29, 1350-1355, (1986).

8- C. Paoletti, S. Cros, N. Dat-Xuong, P. Lecointe, A. Moisand, Chem. Biol. Interact., 25, 45 (1979).

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