Biochemical Pharmacology, Vol. 31, No. 12, pp. 2161–2167, 1982.
Printed in Great Britain.

0006-2952/82/122161-07 $03.00/0

MITOCHONDRIAL AND NUCLEAR MUTAGENICITY OF ELLIPTICINE AND DERIVATIVES IN THE YEAST *SACCHAROMYCES CEREVISIAE*

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(*Received* 18 *May* 1981; *accepted* 25 *January* 1982)

Abstract—Haploid strains of the yeast *Saccharomyces cerevisiae* were tested for their sensitivity to ellipticine and nine derivatives; some of them exhibiting antitumor properties. Different mutagenic properties of ellipticines are described. Charged ellipticines, which were ineffective in Ames' bacterial assay, were found to be potent inducers of the mitochondrial ρ^- mutation: induction proceeded even in the absence of growth. Uncharged ellipticines increased mitochondrial antibiotic resistance mutations, whereas charged derivatives decreased them. Ellipticine derivatives enhanced, reduced, or did not change the reversion frequencies of nuclear auxotrophic markers. The result depended on the strain being tested: no structure–effect relationship existed. As some ellipticine derivatives were mutagenic in *Saccharomyces cerevisiae* despite being ineffective in Ames' assay, multiple tests should be used to check that chemotherapeutic drugs are devoid of mutagenic properties.

Derivatives of ellipticine (5,11-dimethyl-(6H)-pyrido[4,3-b]carbazole) have some activity against experimental [1, 2] and human [3] tumors. Substances which might be useful in the treatment of human cancer must be screened for their genetic effects as well as for their chemotherapeutic and toxic properties. The mutagenicity of chemotherapeutic agents [4] could be studied using Ames' bacterial assay [5]. This test has been used by Lecointe *et al.* [6] for checking the mutagenicity of ellipticine derivatives in the search for derivatives devoid of a mutagenic effect. The yeast *Saccharomyces cerevisiae* has been used for studying the mutagenic effects of many drugs like acridines, actinomycin D, adriamycin, bleomycin and cyclophosphamide [7–10]. Some mutagens require, in Ames' bacterial test, an activation by liver microsomes containing cytochrome P-450 which is naturally present in yeast [11, 12]. The multiple genetic properties of *Saccharomyces cerevisiae* make it a good tool for studies on the mutagenic effects of ellipticines. Three approaches were used. The first was based on the fact that intercalating dyes, like acridines and ethidium bromide, are known to induce ρ^- mutants [13, 14], which have impaired mitochondrial respiration [15] following a specific mitochondrial DNA degradation [16]; the question raised was whether the ellipticines, intercalating dyes [17], owed this ρ^- inducing power. The second, was designed to check if ellipticines induced chloramphenicol resistance, which is known to be a specific mitochondrial mutation in yeast [18]. A final way of analysing mutagenic effects was to measure the reversion of nuclear auxotrophic markers.

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MATERIALS AND METHODS

Strains

The genotypes of the four *Saccharomyces cerevisiae* strains used in this work are given in Table 1: a and α are mating type; Ade, His, Ura and Trp are auxotrophic markers for adenin, histidin, uracil and tryptophan; ρ^+ indicates that the strain can grow in

Table 1. Genotypes of the 4 yeast strains and their phenotype in presence of 9-methoxy-ellipticine in fermentable and respirable media

	Genotype		Carbon source	
Strain	Nuclear	Mitochondrial	Glucose	Glycerol Lactate Ethanol
IL 88D	a Ura^-	$\rho^+\omega^+$ $C^RE^RO^S$	R	S
DP_1-1B/7	α His^- Trp^-	$\rho^+\omega^+$ $C^SE^SO^R$	R	S
DG3-58	α His^-	$\rho^+\omega^+$ $C^SE^RO^R$	R	S
$14/1/R_9$	a Ade^- His^- Ura^-	$\rho^+\omega^+$ $C^SE^SO^S$	R	R

The meaning of genotype abbreviations is given in Materials and Methods.
R and S stand for resistance, or sensitivity, to 9-methoxyellipticine, i.e. ability to grow in solid media containing 80 μM of this drug, compared to the control without the drug, after 4 days of incubation.

fermentable and respirable media (ρ^- mitochondrial respiratory deficient mutants can, by contrast, only grow in fermentable medium); ω^+ indicates the polarity of mitochondrial recombination [19]. C, E and O indicate the mitochondrial resistance (R) or sensitivity (S) of the strains to chloramphenicol, erythromycin and oligomycin antibiotics. The strains were kindly supplied by M. Gouhier, E. Moustacchi and P. P. Slonimski.

Media

Solid media. Different carbon sources are added to a same basal medium: yeast extract (Difco Laboratories, Detroit, MI) 1%; bacto-peptone (Difco) 1%; Na/K phosphate buffer 0.1 M, pH 6.5; bacto-agar (Difco) 2%.

Carbon source:	Glc	Gly	Lact	Eth	D
Glucose (%)	2	—	—	—	0.1
Glycerol (%)	—	2	—	—	2
Lactate (%)	—	—	2	—	—
Ethanol (%)	—	—	—	2	—

Gly–O: standard Gly medium plus 3 μg/ml of oligomycin; Gly–C, standard Gly medium plus 4 mg/ml of chloramphenicol; Wo, yeast nitrogen base (0.67%), glucose (2%), Na/K phosphate buffer 0.1 M pH 6.5 (minimal medium only allowing the growth of prototroph yeast strains); Wo AH, Wo medium plus adenine 20 μg/ml and histidine 10 μg/ml.

The drugs (ellipticine derivatives or antibiotics) were added to the sterilized medium at 60° and the medium then cooled down.

Liquid media. Glucose 2% and glycerol 2%, as carbon source, were used with the same basal medium devoid of agar. Cultures were performed at 28°. Replica plating technique was used according to Lederberg and Lederberg [20].

Measurement of yeast cell respiration

A culture of yeast strain IL 88D was grown in glycerol 2% liquid medium. Cells were harvested during the exponential phase, washed, and concentrated to 20 mg/ml of cellular proteins in Na/K phosphate buffer, 0.1 M (pH 6.5). The respiration rate of concentrated cells was determined by polarographic measurement on a Gilson oxygraph model KM with a Clark type oxygen electrode. The experiment was carried out at 28° in a 2 ml chamber, in a medium containing Na/K phosphate buffer, 0.1 M (pH 6.5), and ethanol, 27 mM.

Protein concentration was measured by the method of Stickland [21]; 1 mg of cellular proteins corresponds to 7×10^7 cells in our culture conditions. Cells were counted in a hemocytometer.

Selection of antibiotic resistant mutants, prototroph revertants, ρ^- mutants and determination of viable cells

The experimental procedure is described in Fig. 2. The four strains were replicated in glucose solid media, with or without ellipticines, and allowed to grow for 4 days at 28°. Each spot strain was suspended in cold distilled water.

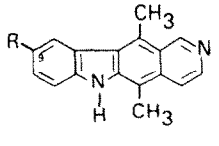
Antibiotic resistant mutants. 0.2 ml of the yeast suspension was plated in Gly-O for strain IL 88D, and Gly-C, for strains DP1-1B/7, DG3-58 and 14/1/R_9. Resistant colonies were counted after 10 days at 28°.

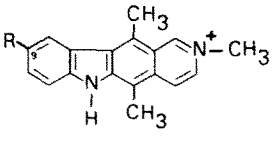
Prototroph revertants. 0.2 ml of the yeast suspension was plated in Wo, for strains IL 88D and DG3-58, (selection of uracil$^+$ and histidine$^+$ revertants, respectively), and in WoAH for strains DP1-1B/7 and 14/1/R_9 (selection of tryptophan$^+$ and uracil$^+$ revertants, respectively). Colonies were counted after 10 days at 28°.

ρ^- Percentage and number of viable cells. The yeast suspension was diluted and plated in D medium. After 4 days at 28° the 'petites' and 'grandes' colonies were counted for ρ^- percentage determination [22]. After 4 days we also checked for 'sectored colonies' (mixing of ρ^+ and ρ^- cells) by the tetrazolium (TTZ) overlay procedure [23]. The total colonies (ρ^+ and ρ^-) gave the number of viable cells.

Drugs

Drugs were kind gifts of N. Dat-Xuong (C.N.R.S., Gif-sur-Yvette, France) and P. Lecointe (C.N.R.S., Toulouse, France). The purity of all the compounds was checked by high performance liquid chromatography [24]. The structure of ellipticine and derivatives is given in Fig. 1. All, except for the 9-bromo-ellipticine derivative, intercalated into DNA [17].





	R		R
ELLIPTICINE	H	2-METHYL-ELLIPTICINIUM	H
9-HYDROXYELLIPTICINE	OH	2-METHYL-9-HYDROXYELLIPTICINIUM	OH
9-METHOXYELLIPTICINE	CH_3O	2-METHYL-9-METHOXYELLIPTICINIUM	CH_3O
9-BROMOELLIPTICINE	Br	2-METHYL-9-BROMOELLIPTICINIUM	Br
9-AMINOELLIPTICINE	NH_2	2-METHYL-9-AMINOELLIPTICINIUM	NH_2

Fig. 1. Structure of ellipticine and derivatives.

Table 2. Percentage of ρ^- mutants induced by 80 μM ellipticine derivatives in solid glucose medium

Drug	Strain	IL 88D	DP1-1B/7	DG3-58	14/1/R$_9$
Control		6.7	6.0	2.2	0.8
Ellipticine		1.9	1.0	0.8	0.6
9-Hydroxyellipticine		9.7	6.4	0.8	1.5
9-Methoxyellipticine		4.8	1.8	2.0	0.9
9-Bromoellipticine		4.0	2.1	1.7	2.1
9-Aminoellipticine		7.9	8.9	3.4	0.2
2-Methyl-ellipticinium		96.1	75.2	67.0	1.2
2-Methyl-9-hydroxyellipticinium		54.4	63.0	17.2	3.2
2-Methyl-9-methoxyellipticinium		34.2	22.3	12.9	0.9
2-Methyl-9-bromoellipticinium		14.2	7.6	2.9	0.7
2-Methyl-9-aminoellipticinium		2.6	2.0	1.4	0.5

RESULTS

Selection of yeast strains sensitive to ellipticine derivatives

9-Methoxyellipticine was used to test the ability of 24 haploid yeast strains to grow in the four carbon sources chosen: three respirable and one fermentable. This drug was selected because it was the first of the ellipticine series to be employed against human cancers [3]. A concentration of 80 μM was found to be most suitable. A strain is defined as sensitive when it gives no spots in the agar medium containing 9-methoxyellipticine after 4 days at 28°. In the glucose solid medium, all 24 strains were resistant; the spots were of the same size in the presence, or in the absence of the drug. Three strains were, however, sensitive to the drug in respirable media, i.e. glycerol, lactate or ethanol. We retained these three sensitive strains and a resistant one for the further studies (Table 1). Their sensitivity to nine compounds belonging to the ellipticine series (Fig. 1) was checked. In glucose solid medium the four strains were resistant. The growths of the 9-methoxyellipticine sensitive strains (IL 88D, DP1-1B/7 and DG3-58) were selectively inhibited in respirable media by ellipticine derivatives (except 9-hydroxyellipticine and 9-aminoellipticine, with no effect); the 9-methoxyellipticine resistant strain (14/1/R$_9$) was resistant to all compounds except ellipticine itself.

ρ^- Induction by ellipticine derivatives in glucose solid medium

The ρ^- cytoplasmic 'petite' mutation, occurring spontaneously at high frequency in yeast, is accompanied by the loss of the respiratory functions

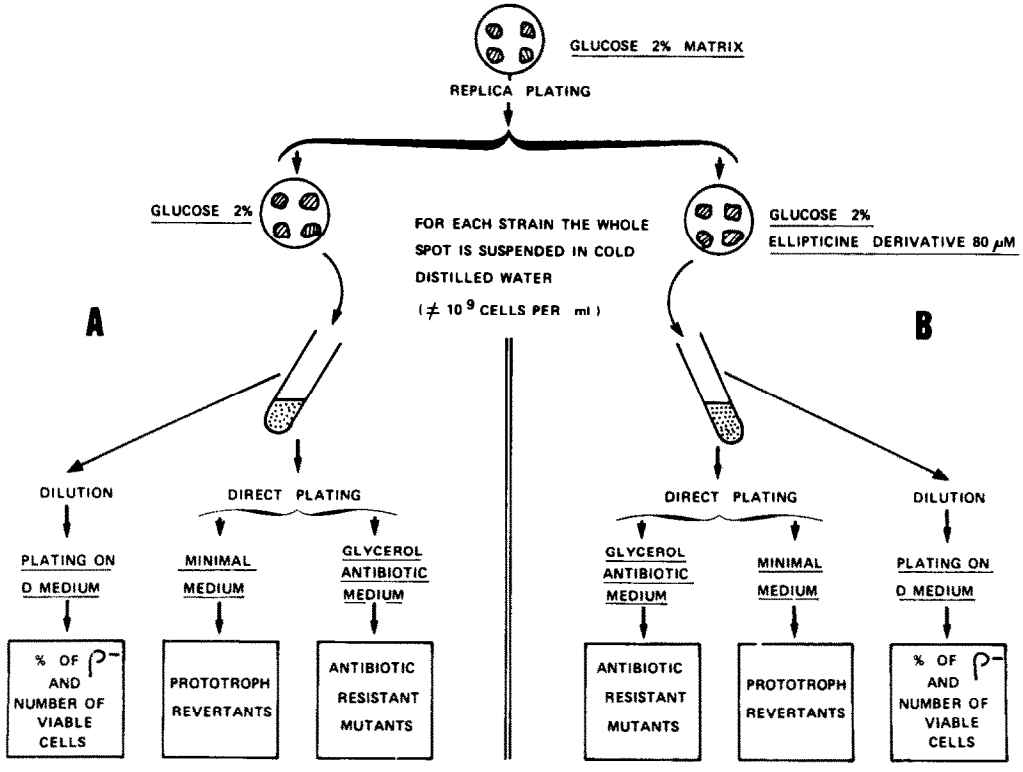


Fig. 2. Schematic representation of mutagenesis process in glucose solid medium and results analysis.

of the cells [15]. If ellipticine derivatives were inducers of this mutation, this would explain the inability of yeast strains to grow in the presence of most of them in respirable media. The percentages of ρ^- 'petites' exposed in Table 2 were obtained after a 4 days treatment with 80 μM of drug. The mutagenesis process and analysis of results are described in Fig. 2. None of the five uncharged derivatives induced ρ^- mutants in any of the four strains tested. The charged ellipticines (except 2-methyl-9-amino-ellipticinium) by contrast, induced ρ^- mutants: in the case of 2-methyl-ellipticinium, transforming all IL 88D cells into ρ^-. There were no mixed colonies (checked by the tetrazolium overlay procedure). Another charged derivative, the 2-methyl-9-azide ellipticinium induced ρ^- mutants in strain IL 88D (result not shown). Sensitive (IL 88D, DP1-1B/7, DG3-58) and resistant (14/1/R_9) strains were distinguished by their ability to grow in respirable solid media in the presence of 9-methoxyellipticine. ρ^- Inductions were only found in the sensitive strains. The ρ^- induction could explain this sensitivity only in two cases: IL 88D and DP1-1B/7 with 2-methyl-ellipticinium derivative. Nevertheless IL 88D, DP1-1B/7 and DG3-58 whose growth was selectively inhibited by ellipticine, 9-methoxyellipticine and 9-bromoellipticine in respirable media, produced no ρ^- induction with any of these compounds. However, we found that these three derivatives and the 2-methyl-9-bromoellipticinium one, were potent

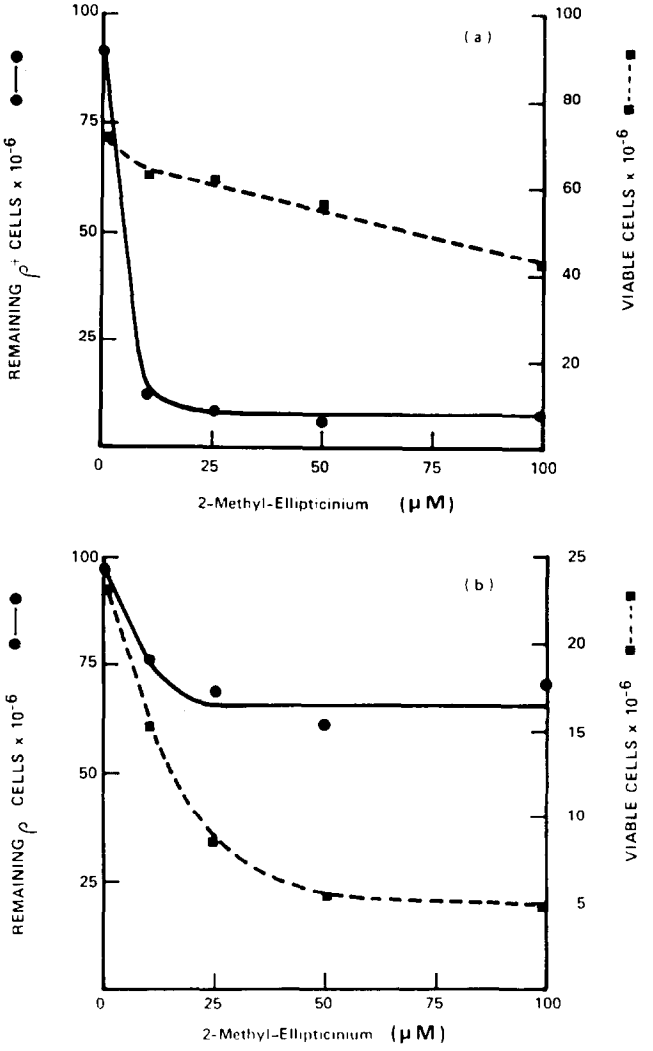


Fig. 3. ρ^- mutagenesis as a function of 2-methyl-ellipticinium concentration in IL 88D yeast strain. (a) ρ^- Induction during vegetative growth: flasks of 100 ml containing 10 ml of glucose 2% medium with phosphate buffer 0.1 M pH 6.5 are inoculated at 10^7 cells/ml, and drug then added. After 7 hr of aerobic growth at 28°, samples are diluted and plated in D medium. ρ^+ and ρ^- colonies are counted after 4 days. (b)ρ^- Induction under non growing conditions: a culture of the strain is performed at 28° in glucose 2% medium. Cells are harvested towards or at the end of exponential growth, washed and suspended in phosphate buffer 0.1 M pH 6.5 for 1 hr starvation at 28°. Then 100 ml flasks containing 10 ml of buffer are inoculated with the starved cells at 2×10^7 cells/ml. Drug is added. After 7 hr of stirring at 28°, samples are diluted and plated in D medium. ρ^+ and ρ^- colonies are counted after 4 days.

inhibitors of oxygen consumption of yeast cells (data not shown) as already described for ellipticine in Ehrlich ascite cells respiration [25].

It is well known that two kinds of ρ^- induction exist: the first, is exemplified by drugs like acridine which induces mutation only during vegetative growth [13], while the second occurs with drugs like ethidium bromide, which transforms the mother cells into ρ^-, even in absence of growth [14]. Tests were made to determine to which type ellipticine belonged, by measuring mutagenesis during vegetative growth, and in the absence of growth, in liquid media. The couple drug/strain 2-methyl-ellipticinium/IL 88D was chosen since it was the one which exhibited the highest ρ^- induction in solid medium (Table 2).

ρ^- Mutagenesis as a function of 2-methyl-ellipticinium concentration

Growing conditions. Figure 3a shows the effect of 7 hr treatment (compared to 4 days on solid medium); even at the lowest concentration, the drug converted practically all of ρ^+ cells to ρ^- mutants. The number of total colonies (ρ^+ plus ρ^-) gives the number of viable cells; 2-methyl-ellipticinium decreased this number from 7.22×10^7 cells/ml in the control to 4.18×10^7 cells/ml with 100 μM of drug. The number of cells recovered was nonetheless higher than the number inoculated. The reduction might, therefore, be due to the slight inhibition of growth met in glucose liquid culture of IL 88D, in presence of the drug, rather than to a direct cytotoxic effect. Ellipticine ρ^- induction resulted from events occurring during the 7 hr of culture in liquid medium, rather than during the 4 days of growth in D medium used for revealing ρ^+ and ρ^- colonies: no ρ^- induction occurred if a sample of culture was plated in D medium immediately after the addition of the drug.

Non-growing conditions. 2-Methyl-ellipticinium was seen to induce ρ^- mutants even in the absence of growth (Fig. 3b) and thereby to resemble ethidium bromide. Slonimski *et al.* [14] found that ethidium bromide converted all the ρ^+ cells into ρ^- mutants without any cell mortality. In our experiment with a 100 μM drug, ρ^- induction did not exceed 30%, and was accompanied by a marked loss of cell viability (only 5.33×10^6 cells/ml instead of 23.20×10^6 cells/ml in control). The 2-methylellipticinium derivative could probably kill the cells directly without first converting them to a ρ^- state. A sample of culture plated in D medium immediately after addition of the drug, again showed no ρ^- induction or cytotoxicity.

ρ^- Mutation resulting from gross mitochondrial DNA alteration [26] is specific for yeast, facultative aerobic eukaryote, and does not exist in the strictly aerobic human or mouse cells. Drugs which induce ρ^- mutation, nonetheless, can affect such cells, and always have the mitochondrial DNA as target: even if ethidium bromide does not provoke the mitochondrial DNA degradation in cultured mouse cells [27] in the same way as in yeast [16, 28], it causes a rapid inhibition of mitochondrial DNA synthesis, and induces a stepwise increase in the negative superhelix density of the pre-existing closed circular mitochondrial DNA [29].

The mutagenic effects of ellipticines resulted in punctual mutations in Ames' test [6]. We, therefore, investigated the punctual mitochondrial and nuclear mutations induced by ellipticines, by examining mitochondrial antibiotic resistant mutations and nuclear auxotrophic marker reversions.

Punctual mitochondrial mutagenesis. Coen *et al.* [18] demonstrated that resistance to chloramphenicol in *Saccharomyces cerevisiae* results from mitochondrial punctual mutations. It should, therefore, be possible to test the punctual mitochondrial mutagenic action of a drug by studying the appearance of spontaneous chloramphenicol resistant mutants in certain yeast strains. The strain IL 88D is already resistant to chloramphenicol, so oligomycin resistant mutants were looked for. With 3 μg/ml of oligomycin, the majority of resistant mutants are mitochondrial.

Mutation frequencies were established from the ratio between the number of resistant colonies in antibiotic media (Gly-O and Gly-C) and the number of ρ^+ viable cells determined in D medium. We can only relate to ρ^+ cells because ρ^- cells cannot grow in glycerol media. The ratio between the frequencies of drug induced mutations and spontaneous muta-

Table 3. Oligomycin and chloramphenicol resistant mutants induced by 80 μM ellipticine derivatives: factors of enhancement

Drug \ Strain	IL 88D $O^S \rightarrow O^R$	DP1-1B/7 $C^S \rightarrow C^R$	DG3-58 $C^S \rightarrow C^R$	14/1/R_9 $C^S \rightarrow C^R$
Ellipticine	1	94	1	1
9-Hydroxyellipticine	5	1	1	1
9-Methoxyellipticine	16	6	1	1
9-Bromoellipticine	1	1	1	1
9-Aminoellipticine	12	1	1	1
2-Methyl-ellipticinium	1	1	1/536	1/3
2-Methyl-9-hydroxyellipticinium	1/8	1/35	1/79	1
2-Methyl-9-methoxyellipticinium	1	1	1	1
2-Methyl-9-bromoellipticinium	1/250	1	1	1
2-Methyl-9-aminoellipticinium	1/13	1	1	1

Factors of enhancement represent the ratio between the frequencies of mutants arising in the presence of drug and spontaneous mutants. Factor 1 indicates no significant difference in the 2 frequencies.

tions, are given in Table 3. Three types of result were obtained; no drug effect (ratio = 1), a mutagenic effect varying from 5 to 94 fold (ratio > 1) and a reduced mutation rate varying from 4 to 536 fold (ratio < 1). If the results are analysed by clustering the strains, the ten derivatives could be split into two groups: uncharged ellipticines (except for 9-bromoellipticine which had no effect) exhibited a mutagenic effect, while ellipticinium derivatives (with the exception of 2-methyl-9-methoxyellipticinium which had no effect) reduced the spontaneous mutation rate. This last effect, called antimutagenesis, has been widely studied in several microbial systems [30]. An anti-mutagenic effect of many drugs (acridines, quinacrine, actinomycin D) has been described with *Saccharomyces cerevisiae* tests for nuclear markers. Punctual mitochondrial mutagenesis has been shown to result from uncharged ellipticines which were unable to induce ρ^- mutants. Even when charged ellipticines induce ρ^- mutants, they also act on the remaining ρ^+ cells by reducing punctual mitochondrial mutation: 2-methylellipticinium in the strain DG3-58 induced 67% of ρ^- (Table 2); the 33% of remaining ρ^+ cells give rise to less chloramphenicol resistant mutants than the control (Table 3).

The two types of mutation studied, ρ^- and antibiotic resistance are mitochondrial. As the four strains bore nuclear auxotrophic markers, it was possible to investigate nuclear mutagenic effects of ellipticines.

Nuclear mutagenesis

Reversion frequencies were determined from the number of prototroph colonies in minimal media (Wo and Wo AH) compared to the number of total viable cells (ρ^+ and ρ^-) established by the D medium analysis because both ρ^+ and ρ^- prototrophs can grow in Wo or Wo AH. The three kinds of results already described for mitochondrial punctual mutations were also found for auxotrophic nuclear reversions (Table 4). Both the factors of mutagenesis, which varied from 7.5 to 2650, and the factors of antimutagenesis, which varied from 4 to 7300, were more important than mitochondrial markers. Mitochondrial markers however enabled a classification of the derivatives into two groups: charged and uncharged molecules. With nuclear markers this was not possible, as the same derivative enhanced, decreased, or was without effect on reversion frequency, depending on the strain tested.

DISCUSSION

The search for derivatives devoid of a mutagenic effect, led Lecointe *et al.* [6] to observe that quaternarized ellipticines were ineffective in Ames' test, although Ames' strains have been stripped of their lipopolysaccharide layer for greater permeability. Four of the five charged ellipticines tested in this study, were found to be potent ρ^- inducers in the strains defined as sensitive. The electric charge seems to be a prerequisite for this induction, as another charged derivative, the 2-methyl-9-azide ellipticinium, also induced ρ^- mutants. It should be recalled that ethidium bromide, a highly active ρ^- inducer, is also a charged molecule [14]. 2-Methyl-ellipticinium was like this drug, as it too induced ρ^- cells in the absence of growth, even if it was cytotoxic under these non-growing conditions. In view of the results observed on punctual mitochondrial mutation, ellipticines could be classified into charged and uncharged molecules: uncharged derivatives increased the frequencies of arising chloramphenicol resistant mutants, and charged ellipticines reduced these frequencies. At the nuclear level, all the ellipticines were effective but reversion of auxotrophic markers was increased, or reduced, depending on the strain tested.

The mitochondrial and nuclear mutagenic process did not seem to be related: in strain DP1-1B/7, ellipticine itself increased the chloramphenicol resistant mutants 94 fold, but had no effect on the reversion rate of tryptophan auxotrophic marker; furthermore, the 2-methylellipticinium derivative which did not affect the frequency of chloramphenicol resistant mutants, increased 40 fold tryptophan$^+$ reversion in the same strain.

An interesting point is raised by the mutagenic effects of 9-bromoellipticine; although, it does not intercalate in DNA, it exhibits both nuclear mutagenic and antimutagenic properties, according to the strain tested: it enhanced uracil$^+$ revertants in the strain 14/1/R$_9$ 7.5 fold, yet it reduced them in strain IL 88D 80 fold. It is also remarkable that 9-bromoellipticine and the equivalent-charged

Table 4. Prototroph revertants induced by 80 μM ellipticine derivatives: factors of enhancement

Drug \ Strain	IL 88D Ura$^-$ → Ura$^+$	DP1-1B/7 Trp$^-$ → Trp$^+$	DG3-58 His$^-$ → His$^-$	14/1/R$_9$ Ura → Ura$^-$
Ellipticine	1	1	1/98	1
9-Hydroxyellipticine	1	707	not done	1
9-Methoxyellipticine	1/144	1	1/4	1
9-Bromoellipticine	1/80	1	1	7.5
9-Aminoellipticine	484	18	1	1/1400
2-Methyl-ellipticinium	1	40	1/6	1
2-Methyl-9-hydroxyellipticinium	1	1	1/42	365
2-Methyl-9-methoxyellipticinium	1/13	1/68	1/11	192
2-Methyl-9-bromoellipticinium	1/7300	1	3	1315
2-Methyl-9-aminoellipticinium	1/6	1	4	2650

See legend of Table 3.

derivative, 2-methyl-9-bromoellipticinium which intercalates in DNA, are correlated in their mutagenic effects in strain 14/1/R_9 and in their antimutagenic effects in strain IL 88D (Table 4). If intercalation into DNA does not seem necessary for mutagenesis or antimutagenesis, it should not be forgotten that 9-bromoellipticine is a powerful mutagen in Ames' test, and even if it does not intercalate in DNA, it has an affinity for the outer binding sites of DNA [31].

The antimutagenic effects of ellipticines were found with a mitochondrial-arising mutation and also with a nuclear reversion. Antimutagenesis with many drugs have been studied in *Saccharomyces cerevisiae* with different nuclear mutations: 5-amino-acridine (51 μM) reduces mutation rate from canavanine sensitivity to resistance 30 fold, and histidine$^+$ reversion 6 fold [7], actinomycin D (38 μM) (ineffective in Ames' test, [4]) reduces canavanine sensitivity to resistance 30 fold [8]. These drugs exhibit antimutagenic properties during vegetative growth like ellipticines, and at similar concentrations.

In conclusion, the fact that charged ellipticines, like the antitumor antibiotics actinomycin D [8] and anthramycin [32] are ineffective in the Ames' test, yet exhibit mutagenic and antimutagenic effects in *Saccharomyces cerevisiae*, underlines the need for multiple tests for drug mutagenicity studies and for the search for drugs devoid of mutagenic properties.

Acknowledgements—We are indebted to G. Muzard (Institut Gustave Roussy) for conducting the high performance liquid chromatography of the drugs and to E. Franque for expert technical assistance.

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