Biochemical Pharmacology, Vol. 27, pp. 1203–1209.
© Pergamon Press Ltd. 1978. Printed in Great Britain.
0006-2952/78/0415-1203$02.00/0

ELLIPTICINES AS POTENT INHIBITORS OF ARYL HYDROCARBON HYDROXYLASE: THEIR BINDING TO MICROSOMAL CYTOCHROMES P450 AND PROTECTIVE EFFECT AGAINST BENZO(a)PYRENE MUTAGENICITY

PIERRE LESCA,* PIERRE LECOINTE,* CLAUDE PAOLETTI*
and DANIEL MANSUY†

* Laboratoire de Pharmacologie et de Toxicologie Fondamentales 205 route de Narbonne, 31078 Toulouse Cedex, France
† Laboratoire de Chimie, Ecole Normale Supérieure 24 rue Lhomond, 75231 Paris cedex 05, France

(*Received* 16 *September* 1977; *accepted* 8 *November* 1977)

Abstract—Ellipticine (5,11-dimethyl-6-H-pyrido[4-3b]carbazole) and its derivatives bind strongly to the oxidized and reduced liver microsomal cytochromes P450 of differently pretreated rats, producing typical difference spectra, with peaks respectively at 428 (ox.) and 445 nm (red.) (with spectral dissociation constants around 10^{-6} and 10^{-5} M, respectively). The high affinity of ellipticine for microsomes containing a great proportion of cytochrome P448, explains its role of strong inhibitor of benzo(a)pyrene hydroxylase. Accordingly, we found a good correlation between the binding properties of the ellipticines and their inhibitory effect upon: (1) the microsomal formation of water soluble metabolites of benzo(a)pyrene, (2) the covalent binding of reactive metabolites of this hydrocarbon to DNA, and (3) the mutagenic activity of benzo(a)pyrene in the *Salmonella typhimurium* test.

A new approach and a promising way to control chemical carcinogenesis is to prevent the original carcinogenic injury to the cell. It is well established now that the enzyme system which metabolizes a wide variety of exogenous compounds (drugs, hydrocarbons, pesticides, etc.) in view of their detoxification plays also a key role in the activation of a great number of chemicals to carcinogenic intermediates [1, 2]. This biotransformation consists of an oxidation mediated by cytochrome P450, the different forms of which are naturally present or chemically induced in 90 per cent of the tissues [3–5].

A great number of compounds exist which modify, depress or inhibit the rate of drug metabolism [6]. Among them, some exert so large an inhibitory effect that the metabolism, mutagenicity and carcinogenicity of compounds such as polycyclic hydrocarbons can be strongly decreased. 7,8-Benzoflavone which belongs to this class of very potent inhibitors of aryl hydrocarbon hydroxylase is able to block 3-methylcholanthrene mutagenesis *in vitro* or oncogenic transformation of mouse cells in culture and to inhibit 7,12-dimethylbenz (a) anthracene tumorigenesis in mice [7–11]. It was interesting to examine whether ellipticines, which have a polycyclic aromatic structure (Fig. 1) and a basic nitrogen at position 2, could bind to cytochrome P450 like other pyridinic compounds, and exhibited any inhibitory effect on the metabolism of a known cancerogenic compound.

This paper concerns the interaction of several derivatives of ellipticine with rat liver cytochromes P450 and the consequences of the observed strong binding of ellipticine to cytochrome P448-Fe(II). The metabolism of benzo(a)pyrene, the covalent binding of its metabolites to DNA and its mutagenic activity in the *Salmonella*/microsomes test system developed by B. N. Ames [12] are, in fact greatly decreased by ellipticine. These results, based upon the fundamental data of interaction with P450-cytochromes, demonstrate the potent antimutagenic role of ellipticines.

MATERIALS AND METHODS

Ellipticine and its 9-methoxy and 6-methyl derivatives were prepared according to the method of Dalton [13]. The syntheses of 9-hydroxy and 9-aminoellipticine have been already described [14]. 6-Methyl-9-bromoellipticine resulted from the reaction of 6-methylellipticine with bromine in glacial acetic acid (P. Lecointe, personal data). 2-Methylellipticinium iodide was prepared by refluxing ellipticine with methyl iodide in methanol; its structure was confirmed by NMR spectroscopy*. 9-Hydroxy-2-methylellipticinium acetate was a kind

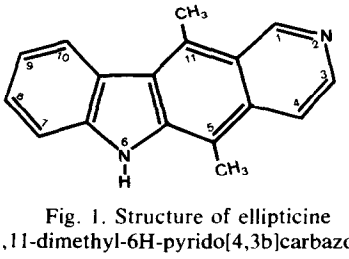

Fig. 1. Structure of ellipticine (5,11-dimethyl-6H-pyrido[4,3b]carbazole).

* *Abbreviations*—NMR spectroscopy, nuclear magnetic resonance spectroscopy; TLC, thin layer chromatography; MP, metyrapone; 7,8-BF, 7,8-benzoflavone; PB, phenobarbital; BP, benzo(a)pyrene; TMS, buffer pH 7.5 containing 50 mM Tris-HCl, 3 mM $MgCl_2$ and 200 mM sucrose; DMF dimethylformamide.

gift of Dr Nguyen Dat Xuong. The purity of these compounds was checked by TLC and NMR spectroscopy and found to be better than 95 per cent. Aroclor 1254, a polychlorinated biphenyl mixture, was a gift of Dr. M. Hofnung. Metyrapone (MP) was obtained from Ciba–Geigy, Basel, Switzerland. 7,8-Benzoflavone (7,8-BF) was purchased from Prolabo, Paris, France. Calf thymus DNA was obtained from Sigma Chemical Co., St Louis, MO.; it was purified by phenol treatment. [^{3}H]benzo(a)pyrene (1 Ci/m-mole) was purchased from C.E.A., Saclay, France; its radiochemical purity was controlled by TLC on alumina gel plates using benzene–cyclohexane (50:50) as ascending solvent.

Male Wistar rats weighing about 150 g were used. When the animals were treated, they received intraperitoneally, (i.p.) either 80 mg/kg phenobarbital (PB) (one dose/day for 3 days) or 20 mg/kg benzo(a)pyrene (BP) or ellipticine [15] dissolved in olive oil (one dose/day for 2 days), or 500 mg/kg Aroclor 1254 (one dose, the animal being killed 5 days after treatment). Livers were removed and the microsomes prepared according to a previously described procedure [15]. The microsomal cytochrome P450 content was determined according to Omura and Sato [16].

The hydroxylation of benzo(a)pyrene was measured according to the method of Nebert and Gelboin [17]. The incubation mixture contained in 1 ml 50 mM Tris–HCl, 3 mM $MgCl_2$ and 200 mM sucrose (TMS), pH 7.5, 1 mg NADPH, 0.025 mg BP (dissolved in 5 μl acetone) and microsomes (0.5 mg protein/ml) if not stated otherwise. The mixture was incubated 10 min. at 37°, if not stated otherwise, and the reaction stopped by addition of 4 ml of 25% acetone in hexane. The subsequent operations were carried out as described [17].

Difference spectra were recorded on a Beckman Acta C-III spectrophotometer using 10 mm cuvettes containing 3 ml of a microsomal suspension in TMS buffer. Ellipticine and derivatives were added in DMF solution.

Covalent binding of BP to DNA was monitored by a radioactive assay described by Grover and Sims [2] and by Gelboin [18].

Mutagenesis experiments were performed essentially as described by Ames [12] on *Salmonella* strain TA 100.

RESULTS

Spectral interactions of the ellipticine derivatives with liver microsomes from control or differently pretreated rats. As shown in Fig. 2, a difference spectrum appears with an isosbestic point at 412 nm upon gradual addition of ellipticine to a suspension of oxidized hepatic microsomes from phenobarbital pretreated rats. This type II difference spectrum [5], exhibits a peak at 428 nm and a trough at 405 nm. It is similar to those produced by compounds containing lone pair electrons on nitrogen atoms [19], and should correspond to the binding of the ellipticine pyridinic nitrogen to the sixth coordination site of cytochrome P450-Fe(III). All the tested ellipticine derivatives produce the same type of difference spectrum with a peak at 428 nm and slight

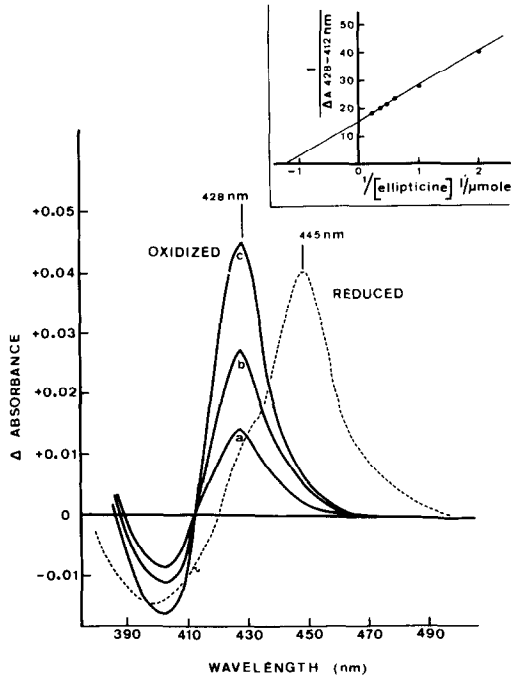

Fig. 2. Substrate binding difference spectra of ellipticine with liver microsomes from phenobarbital-pretreated rats. (—), the oxidized microsomal suspension of 0.55 mg protein/ml (1.32 nmole cytochrome P450/mg protein) in TMS buffer, pH 7.5, was equally divided between 1-cm cuvettes and the baseline recorded. Curves (a), (b) and (c): 0.5, 1 and 3.5×10^{-6} M ellipticine respectively, in the sample. (- - -), $Na_2S_2O_4$ was added prior to the distribution of the same microsomal suspension into the cuvettes. Difference spectrum was obtained after 10 μl addition of a 1.5×10^{-2} M solution of ellipticine to the sample cuvette and 10 μl of solvent (DMF) to the reference cuvette. Inset: Apparent spectral dissociation constants (K_s) of ellipticine with oxidized microsomes (Lineweaver-Burk plots).

variations for the trough and isosbestic point positions (i.e. 9-methoxyellipticine gives a trough at 400 nm and an isosbestic point at 410 nm). Because of their highly polar character, the quaternary ellipticinium salts, 2-methylellipticinium acetate and 9-hydroxy-2-methylellipticinium iodide fail to produce any spectral change when added to the same microsomes. Ellipticine produces this type II spectrum with a peak at 428 nm whichever type of induction is used. However we have observed that the positions of the minima are dependent upon the pretreatment of the rats. For instance, ellipticine difference spectrum of control rat microsomes exhibits two troughs at 408 and 390 nm whereas the difference spectra of PB, BP and Aroclor 1254 induced rat microsomes show one trough at 405, 398 and 398 nm respectively. Similar variations have been previously reported for the interaction of aliphatic amines with cytochromes P450 [20].

Ellipticines have a high affinity for microsomal cytochromes P450, the apparent spectral dissociation constants, K_s being found around 10^{-6} M (Table 1A). Ellipticine itself exhibits the highest affinity for control rat microsomes ($K_s = 4.3 \times 10^{-7}$ M). The modification of the ellipticine nucleus by substitution with polar groups, as an hydroxy or an amino ($K_s = 4.3 \times 10^{-6}$ M) group in the 9-position, does not

Table 1. Spectral data for the interaction of ellipticine and derivatives with oxidized and dithionite reduced liver microsomes of control and pretreated rats

A: oxidized	Microsomes							
	Control		Phenobarbital		Aroclor 1254		Benzo(a)pyrene	
Product	ΔA max./ nmole cyt. P450	K_s (μM)	ΔA max./ nmole cyt. P450	K_s (μM)	ΔA max./ nmole cyt. P448	K_s (μM)	ΔA max./ nmole cyt. P448	K_s (μM)
Ellipticine	0.037*	0.43	0.064	0.90	0.074	0.75	0.056	0.70
9-Methoxyellipticine	—	0.77	0.065	3.30	0.078	1	0.058	0.61
9-hydroxyellipticine	—	0.65	—	3	—	—	—	0.52
B: reduced								
Ellipticine	0.030†	8.30	0.054	33	0.069		1.30	
9-Methoxyellipticine	—	—	0.056	15	0.070		2.20	
9-Hydroxyellipticine	—	—	0.050	37	—		—	

The conditions were as described under Fig. 2. All values are the average of three independent experiments.
* 428–475 nm absorbancy change produced by a saturating ligand concentration.
† 445–490 nm absorbancy maximal change.

diminish greatly the affinity, the hydrophobic character of the entire molecule remaining very high. We must note in this connection, that 9-hydroxyellipticine dissolved in ethyl acetate is not extractable by alkaline solution (1 N NaOH). The characteristics of ellipticine binding to cytochrome P450 resemble those observed by Jefcoate *et al.* [20] for aniline and pyridine. The affinity of the latter substrates for PB induced microsomes is rather weak ($K_s \simeq 10^{-3}$ M). Nevertheless, they exhibit, as ellipticine, a single binding constant contrary to aliphatic amines which show two distinct binding constants [20].

The difference spectra obtained upon addition of ellipticine to dithionite reduced microsomes exhibit a Soret peak at 445 nm (Fig. 2). This is common feature of compounds containing lone pair electrons on nitrogen atoms [21], and corresponds to the binding of their basic nitrogen to the Fe(II) of cytochrome P450. A second peak, seen as a shoulder around 425–430 nm in Fig. 2 is likewise common for these compounds like metyrapone or pyridine [21]. It should be observed that with ellipticine the absorbance at 428 nm was more prominent with Aroclor and BP stimulated microsomes than with PB induced microsomes. A similar shift of the absorbance in the Soret region had been already observed by Jonen *et al.* [21] when comparing the difference absorption spectrum of metyrapone with PB and methylcholanthrene induced microsomes. The affinity of ellipticine for reduced microsomal cytochrome P450 is dependent upon the pretreatment of the rats. As shown in Table 1B ellipticine exhibits the highest affinity ($K_s = 1.3 \times 10^{-6}$ M) for Aroclor induced microsomes and the lowest ($K_s = 3.3 \times 10^{-5}$ M) for PB induced microsomes.

It was interesting to compare the binding properties of ellipticine with those of another pyridinic compound, metyrapone, which is a well known inhibitor of several cytochrome P450 dependent hydroxylations [22]. As indicated in Table 2, ellipticine interacts more specifically with the P448 form which increases after Aroclor or BP pretreatment of the rats while metyrapone preferentially interacts with the P450 form which increases after PB pretreatment [20]. Thus, when passing from PB to Aroclor induced microsomes, the spectral dissociation constant, K_s, for ellipticine is divided by 1.2 in the

Table 2. Comparison of the spectral data for the interaction of ellipticine and metyrapone with oxidized and dithionite reduced liver microsomes of control and pretreated rats

A: oxidized	Microsomes					
	Phenobarbital		Control		Aroclor 1254	
Product	K_s (μM)	ΔA max./ nmole cyt. P450	K_s (μM)	ΔA max./ nmole cyt. P450	K_s (μM)	ΔA max./ nmole cyt. P448
Ellipticine	0.90	0.064*	0.43	0.037	0.75	0.074
Metyrapone	0.67	0.022†	1.00	0.018	2.00	0.014
B: reduced						
Ellipticine	33	0.054‡	8.30	0.030	1.30	0.069
Metyrapone	2.40	0.051§	16	0.027	390	0.021

All conditions were as described under Fig. 2. All values are the average of three independent experiments.
* 428–475 nm absorbancy change produced by a saturating ligand concentration.
† 425–490 nm absorbancy change produced by a saturating ligand concentration.
‡ 445–490 nm absorbancy change produced by a saturating ligand concentration.
§ 446–490 nm absorbancy change produced by a saturating ligand concentration.

Table 3. Inhibitory effects of ellipticine, metyrapone and 7,8 benzoflavone, added *in vitro*, on benzo(a)pyrene hydroxylation by microsomes of control and pretreated rats

Compound added to incubation mixture*	Concentration (M)	Microsomes			
		Control	PB	BP	Aroclor 1254
		Inhibition of BP hydroxylation†, per cent			
Metyrapone	10^{-5}	7	54	0	0
7,8 benzoflavone	10^{-5}	14	5	41	54
	10^{-6}	10	3	14	15
Ellipticine	10^{-5}	81	72	92	96
	10^{-6}	50	50	64	49
I_{50} Ellipticine‡		1×10^{-6} M	1×10^{-6} M	6×10^{-7} M	1.1×10^{-6} M

The conditions were as described in Methods. The values are the average of duplicate experiments.

* Compounds were added in DMF solution (5 μl). Addition of equivalent amount of pure DMF does not modify the BP hydroxylation. The results are independent upon order of addition of the compounds.

† BP hydroxylase activities in the absence of inhibitors: 70, 135, 350 and 400 *p*moles/min/mg of protein for microsomes of control, PB, BP and Aroclor 1254 rats respectively.

‡ I_{50} (ellipticine concentration giving 50 per cent inhibition of BP hydroxylation) were determined from inhibition curves obtained with six inhibitor concentrations ranging from 2.5×10^{-7} M to 2×10^{-5} M.

oxidized state and by 25 in the reduced state though, for metyrapone, it is multiplied respectively by 3 and 160. It is noteworthy that the differences in affinity of the two compounds are only important for reduced cytochromes. Moreover, the maximum spectral change (ΔA max./nmole of reduced cytochrome P450) produced by ellipticine and metyrapone, which is related to the amount of reduced cytochrome P450 ligand complex increases for ellipticine and greatly decreases for metyrapone when passing from PB to Aroclor induced microsomes.

The ligands of high affinity for reduced cytochrome P450, like metyrapone [23], are generally able to compete with CO for the sixth coordinate position of the Fe(II) heme. This applies also for ellipticine as found by CO titration experiments of liver microsomal cytochromes P450 of control and differently pretreated rats in the presence of increasing concentrations of ellipticine. The proportion of cytochromes P450 which remains able to bind CO (10^{-3} M) decreased from 100 to about 60–70 per cent, when ellipticine concentration in both cuvettes varies from 0 to 2×10^{-5} M. Up to this concentration the proportion of carboxy-cytochromes P450 does not vary significantly.

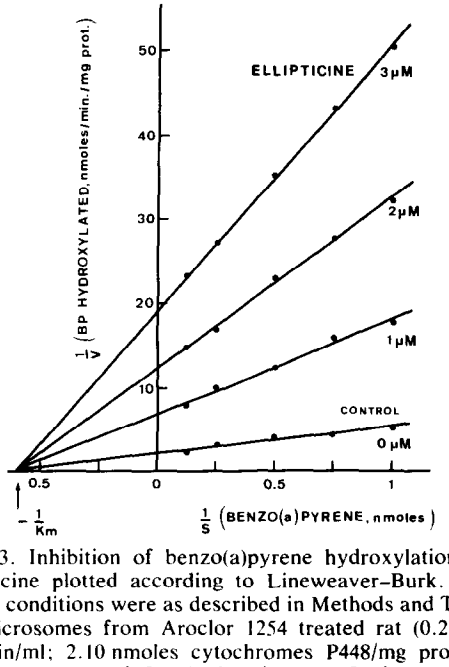

Fig. 3. Inhibition of benzo(a)pyrene hydroxylation by ellipticine plotted according to Lineweaver–Burk. The assay conditions were as described in Methods and Table 3. Microsomes from Aroclor 1254 treated rat (0.25 mg protein/ml; 2.10 nmoles cytochromes P448/mg protein) were used. Incubation time was 5 min.

Inhibition of benzo(a)pyrene hydroxylation by ellipticine. The very high affinity of ellipticine for microsomal cytochromes P450 prompted us to study its possible inhibitory role towards some microsomal hydroxylase activities. As ellipticine presents a high affinity for microsomal cytochrome P448, we tested first its effect on benzo(a)pyrene hydroxylase, a very important system in chemical carcinogenesis. In Table 3, this effect of ellipticine is compared with those of metyrapone and 7,8-benzoflavone which are classical inhibitors respectively of cytochrome P450—particularly the form derived from PB induction [23, 24], and cytochrome P448 [1]—dependent hydroxylations. It appears that ellipticine is a better inhibitor of microsomal BP hydroxylase than 7,8-benzoflavone and metyrapone whichever type of induction is used. BP hydroxylase activity is half decreased by ellipticine concentrations ranging from 6×10^{-7} M to 1.1×10^{-6} M, depending upon the induction. By comparison, 10^{-5} M, 7,8-benzoflavone gives only a 41 and 54 per cent inhibition of the BP hydroxylase activity of microsomes from BP and Aroclor treated rats. Its lower inhibitory power compared to ellipticine is underlined by its I_{50} value of 1.5×10^{-5} M, determined with BP induced microsomes. Metyrapone (10^{-5} M) fails to inhibit the BP hydroxylase activity of BP- and Aroclor-induced microsomes but leads to a 54 per cent inhibition of BP hydroxylase activity of PB induced microsomes (compared with 72 per cent with 10^{-5} M ellipticine).

A Lineweaver–Burk plot treatment (Fig. 3) of the kinetics of the inhibition by ellipticine of the BP hydroxylase activity of Aroclor-induced micro-

Table 4. Inhibitory effect of ellipticine added *in vitro*, on [^{3}H] benzo(a)pyrene covalent binding to DNA by microsomes of control and pretreated rats

Induction of microsomes	Position of the Soret peak after CO titration (nm)	Binding specific activity*	I_{50}† (M)	
			Ellipticine	7,8 benzoflavone
None	450	3.00	5×10^{-6}	—
PB	450	0.83	7.5×10^{-6}	—
Aroclor 1254	448	12.90	2.3×10^{-6}	2.8×10^{-5}
BP	448	13.40	4.5×10^{-6}	—
Ellipticine‡	448	6.10	4.2×10^{-6}	—

The indicated values represent the average of two independent experiments.

* pmoles of [^{3}H] BP metabolites/mg DNA/nmole of cytochrome P450 (or P448).

† concentration giving 50 per cent inhibition of [^{3}H] BP covalent binding to DNA. I_{50} values were determined from inhibition curves obtained with four inhibitor concentrations.

‡ We have formerly shown that ellipticine is an inducer of cytochrome P448 [15].

somes, shows that this inhibition is of the non-competitive type.

Inhibition of benzo(a)pyrene metabolites covalent binding to DNA, by ellipticine. The fluorometric test used in the preceding experiments detects only alkali-extractable metabolites of BP. We then studied the effect of ellipticine on the formation of reactive metabolites of BP, like arene oxides, which are able to bind covalently to DNA. The results shown in Table 4 indicate that very low concentrations (from 2.3×10^{-6} M to 7.5×10^{-6} M) of ellipticine are able to inhibit 50 per cent of the BP covalent binding to DNA. Thus the efficiency of the inhibitor is high whatever the source of microsomes, the smaller effect being obtained with PB induced microsomes. These data correlate well with those of Table 3, 7,8-BF being here also less active (about 10-fold) than ellipticine.

Inhibition of the mutagenicity of benzo(a)pyrene by ellipticines. The preceding results allowed us to believe that the ellipticines, at least those which interact with the catalytic site of cytochrome P450, could reduce the mutagenicity of polyaromatic hydrocarbons. Table 5 shows that ellipticine and 9-hydroxyellipticine inhibit respectively 60 and 78 per cent of the mutagenicity of benzo(a)pyrene, on the *Salmonella* strain TA 100 (Ames' test) when BP and ellipticines concentrations are equal. Ellipticine (and 9-hydroxyellipticine, to a lower extent) is mutagenic by itself. We will discuss later the prospects of finding a non mutagenic inhibitor.

Table 5. Inhibitory effect of ellipticine and 9-hydroxy-ellipticine on mutagenesis by benzo(a)pyrene

Compound added	Concentration (μg/plate)	His$^+$ revertants per plate	
		without BP	with BP (5 μg)
None		0	1126
Ellipticine	2	40	774
	5	184	450
9-Hydroxyellipticine	2	NS*	474
	5	69	249

Experiments were performed on *Salmonella* strain TA 100. The compounds were added to molten top agar containing about 2×10^8 bacteria, a limiting concentration of histidine (7×10^{-5} M) and a trace of biotin (5×10^{-5} M). Then a liver homogenate from Aroclor 1254 induced rats was added, with NADP and G-6P and the mixture was spread over minimal medium Petri plates. Controls included: the number of spontaneous revertants (with and without microsomes) which were subtracted from plate counts, phenotypic characters of the strain, effect of chemicals alone and proficiency of reversion to histidine prototrophy with methyl methanesulfonate.

* Not significantly different from background.

DISCUSSION

As other compounds possessing lone pair electrons on nitrogen atoms, ellipticine is able to bind to the iron of P450-cytochromes. It generally exhibits a high affinity for cytochromes P450, either in the oxidized or in the reduced state. Its affinity for reduced cytochromes is maximum for cytochrome P448. This could explain its inhibitory effect upon microsomal benzo(a)pyrene hydroxylation, covalent binding of reactive metabolites of BP to DNA, and mutagenicity of this cancerogenic hydrocarbon in Ame's test. This also applies to other derivatives such as 9-hydroxy and 9-methoxyellipticine, the former exhibiting a greater protective effect against BP mutagenesis (Table 5). Moreover, the two ellipticinium salts that we have studied did not interact with P450-cytochromes, probably owing to their hydrophilic character, and were also totally devoid of the inhibitory properties of the uncharged molecules.

The large scale and powerful inhibitory properties of ellipticine are most likely due to its strong binding to the heme Fe(II), preventing the fixation and activation of molecular dioxygen. This mechanism is wholly independent of that involved in the effect of ellipticines against L1210 murine leukemia [13, 25, 26] or human cancers [27]. It is not in the authors' intentions to draw any correlation between this cytotoxic antitumor effect, which has not been satisfactorily explained up to now, and the antimutagenic role of ellipticine based on the inhibition of P450-cytochrome functions.

We have shown that ellipticine exhibited a much better inhibitory effect than 7,8-benzoflavone on

BP-hydroxylation (Table 3), and covalent binding of BP metabolites to DNA (Table 4), the I_{50} of ellipticine being in both cases about 10-fold smaller. Moreover, we have observed that ellipticine inhibits the BP hydroxylase activity of Aroclor stimulated microsomes, in a non competitive manner (Fig. 3) though 7,8-BF gives a competitive inhibition of the same activity, as already described by other investigators [28, 29]. This is easily understood when one considers that 7,8-BF binds only to the proteic active site of cytochrome P450 leading to a type 1 spectrum [30], as ellipticine is able to bind strongly through its basic nitrogen to cytochrome P450-Fe(II). Accordingly, 7,8-BF only competes with the substrate (BP) for its fixation to the active site, while ellipticine competes with oxygen for its fixation to Fe(II) heme. Thus, ellipticines appear as new monooxygenases inhibitors acting similarly to metyrapone by their strong binding to the Fe(II) of cytochrome P450. One could expect, however, that because of their high affinity for all microsomal cytochromes P450, they should have a wider range of action than metyrapone which inhibits more particularly the cytochrome P450-dependent monooxygenases activities of PB induced microsomes. In fact, ellipticine even exhibits a slightly better inhibitory power towards BP hydroxylase activity of BP induced microsomes which is cytochrome P448 dependent.

It appears from data in Table 5 that ellipticine is mutagenic on Ames' strain TA 100; this result could be explained by the formation of reactive intermediates during the hydroxylation of ellipticine in position 9 by rat liver microsomes [31]. However, the mutagenicity of ellipticines can be modulated by substitution. We have observed for instance that substitution of the 9-H of ellipticine by $-OCH_3$ or NH_2 increases its mutagenicity [32]. On the contrary, it is noteworthy that 9-OH-ellipticine (Table 5) is less mutagenic by itself than ellipticine whereas it exhibits a similar affinity for cytochrome P450 and even a better protection effect against BP mutagenesis. Studies are in progress in our laboratory to elucidate the relations between the structure of ellipticines, their inhibitory properties against microsomal monooxygenases and their mutagenesis potency. It would be possible to design an uncharged ellipticine derivative, retaining the P450-cytochromes inhibiting properties, but devoid of mutagenic activity. We have been thus able to find that the new derivative 9-fluoroellipticine has a non significant mutagenicity on strain TA 100, and is a better inhibitor than ellipticine and 9-hydroxyellipticine themselves (data not shown).

The correlations between the spectral interactions data and the inhibitory effects on benzo(a)pyrene mutagenesis by ellipticines, as described in this paper, highlight the possibility of a rational search for antimutagenic compounds. Compounds which have a specific high affinity for some form of cytochrome P450 would be good inhibitors of mutagenesis by those exogenous chemicals which are metabolized by the same cytochrome form to reactive intermediates. In this way, 7,8-BF which binds with high affinity to cytochrome P448 strongly inhibits mutagenesis by BP, because this hydrocarbon is preferentially metabolized by this form of cytochrome [33, 34]. Conversely, metyrapone, which has a strong affinity only for the PB-stimulated form of cytochrome P450 (Table 2), is unable to inhibit the covalent binding of metabolites of BP produced by cytochrome P448 (unpublished observations) to DNA. Ellipticines which exhibit a high affinity towards the different forms of cytochrome P450 are expected to inhibit the microsomes-dependent mutagenicity of a great number of chemicals. This hypothesis is actually under investigation.

Acknowledgements—This work received a financial support from DGRST (contrat no. 757–1372, 10 November, 1975). The authors gratefully acknowledge the expert technical assistance of Mrs Maryse Germanier, Michèle Garès, Claire Lesca and Marcelle Vinial. We thank Dr. Muzard for analytical data on ellipticines.

REFERENCES

1. H. V. Gelboin, N. Kinoshita and F. J. Wiebel, *Fedn. Proc.* **31**, 1298 (1972).
2. P. L. Grover and P. Sims, *Biochem. J.* **110**, 159 (1968).
3. D. M. Jerina and J. W. Daly, *Science, N.Y.* **185**, 573 (1974).
4. P. Sims and P. L. Grover, *Adv. Cancer Res.* **20**, 165 (1974).
5. G. J. Mannering, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds B. N. LaDu, H. G. Mandel and E. L. Way) p. 206. Williams & Wilkins Co., Baltimore (1972).
6. G. J. Mannering, in *Concepts in Biochemical Pharmacology* (Eds B. B. Brodie and J. R. Gilette) p. 452. Springer-Verlag, Berlin (1971).
7. L. Diamond and H. V. Gelboin, *Science, N.Y.* **166**, 1023 (1969).
8. J. S. Felton and D. W. Nebert, *J. biol. Chem.* **250**, 6769 (1975).
9. S. Nesnow and C. Heidelberger, *Cancer Res.* **36**, 1801 (1976).
10. H. V. Gelboin, F. Wiebel and L. Diamond, *Science, N.Y.* **170**, 169 (1970).
11. N. Kinoshita and H. V. Gelboin, *Proc. Natn. Acad. Sci. U.S.A.* **69**, 824 (1972).
12. B. N. Ames, J. McCann and E. Yamasaki, *Mutation Res.* **31**, 347 (1975).
13. L. K. Dalton, S. Demerac, B. C. Elmes, J. W. Loder, J. M. Swan and T. Teitei, *Austr. J. Chem.* **20**, 2715 (1967).
14. N. Dat Xuong, M. T. Adeline, P. Lecointe and M. M. Janot, *C.R. Acad. Sci. Serie D* **281**, 623 (1975).
15. P. Lesca, P. Lecointe, C. Paoletti and D. Mansuy, *C.R. Acad. Sci. Serie D* **282**, 1457 (1976).
16. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
17. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
18. H. V. Gelboin, *Cancer Res.* **29**, 1272 (1969).
19. J. B. Schenkman, H. Remmer and R. W. Estabrook, *Molec. Pharmac.* **3**, 113 (1967).
20. C. R. E. Jefcoate, J. L. Gaylor and R. L. Calabrese, *Biochemistry* **8**, 3455 (1969).
21. H. G. Jonen, B. Hüthwohl and R. Kahl, *Biochem. Pharmac.* **23**, 1319 (1974).
22. K. C. Leibman, *Molec. Pharmac.* **5**, 1 (1969).
23. A. G. Hildebrandt, K. C. Leibman and R. W. Estabrook, *Biochem. biophys. Res. Commun.* **37**, 477 (1969).
24. V. Ullrich, P. Weber and P. Wollemberg, *Biochem. biophys. Res. Commun* **64**, 808 (1975).

25. J. B. Le Pecq, N. Dat Xuong, C. Gosse and C. Paoletti, *Proc. Natn. Acad. Sci. U.S.A.* **71,** 5078 (1974).
26. J. B. Le Pecq, C. Gosse, N. Dat Xuong, S. Cros and C. Paoletti, *Cancer Res.* **36,** 3067 (1976).
27. G. Mathé, M. Hayat, F. De Vassal, L. Schwartzenberg, M. Schneider, J. R. Schlumberger, C. Jasmin and C. Rosenfeld, *Rev. Eur. Etud. clin. Biol.* **15,** 541 (1970).
28. F. J. Wiebel and H. V. Gelboin, *Biochem. Pharmac.* **24,** 1511 (1975).
29. W. F. Benedict, J. E. Gielen and D. W. Nebert, *Int. J. Cancer* **9,** 435 (1972).
30. F. M. Goujon, D. W. Nebert and J. E. Gielen, *Molec. Pharmac.* **8,** 667 (1972).
31. P. Lesca, P. Lecointe, C. Paoletti and D. Mansuy, *Biochem. Pharmac.* **26,** 2169 (1977).
32. P. Lecointe, P. Lesca, S. Cros and C. Paoletti, *Chem.-Biol. Interact.* (in press) (1978).
33. A. Y. H. Lu, W. Levin, S. B. West, M. Jacobson, D. Ryan, R. Kuntzman and A. H. Conney, *J. biol. Chem.* **248,** 456 (1973).
34. D. W. Nebert and J. S. Felton, *Fedn. Proc.* **35,** 1133 (1976).