Inhibition of fungal and mammalian sterol biosynthesis by 2-aza-2,3-dihydrosqualene

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2-Aza-2,3-dihydrosqualene (I) and a quaternary ammonium derivative (II) inhibited ergosterol biosynthesis in cells and cell-free extracts of the pathogenic yeast *Candida albicans* as measured by incorporation of radiolabelled precursors. The compounds inhibited squalene epoxidase and 2,3-oxidosqualene cyclase to varying degrees in microsomes from *C. albicans* and from rat liver. The rat liver epoxidase was 50% inhibited by I at 2.4 μM. In *C. albicans* cells, but not in cell-free extracts, I also inhibited lanosterol demethylation and led to accumulation of an unidentified polar product.

(Candida albicans) *Squalene epoxidase* *2,3-Oxidosqualene cyclase* *2-Aza-2,3-dihydrosqualene* *Ergosterol* *Cholesterol*

1. INTRODUCTION

The cyclization of squalene to lanosterol involves sequential action of two enzymes, squalene epoxidase (EC 1.14.99.7) and 2,3-epoxysqualene lanosterol cyclase (EC 5.4.99.7). Compounds such as 2-aza-2,3-dihydrosqualene (I) and 1,1′,2-tris-*nor*-3-trimethylammonium squalene iodide (II) were designed by Cattel and co-workers [1,2] as analogues of a carbocationic high energy intermediate of the cyclization. Compounds I and II inhibited cyclases from higher plants and from rat liver [3] but effects on other enzymes have not been reported. Since I and II are squalene analogues they may also inhibit squalene epoxidase. The latter enzyme is of considerable practical interest as the target of a new class of antimycotic agents, the allylamines [4,5]. Surprisingly, the thiocarbamate antifungals have also been found to inhibit squalene epoxidase [6]. In this report we show that I and II inhibit both squalene epoxidase and the cyclase in microsomes from the pathogenic yeast *Candida albicans,* and from rat liver.

2. EXPERIMENTAL

Compounds I and II were a gift from Professor L. Cattel.

Microsomal squalene epoxidase was assayed as described in [5] from rat liver and in [7] for *C. albicans.* The cyclase was measured by conversion of ^{14}C-labelled 2,3-epoxidosqualene to sterols as described in [8]. Ergosterol biosynthesis in whole cells of *C. albicans* was assayed by incubation with [^{14}C]acetate, extraction of the non-saponifiable lipids and separation by thin layer chromatography as described [8,9]. Sterol biosynthesis in cell-free extracts of *C. albicans* was similarly measured by incorporation of [2-^{14}C]mevalonate [9].

3. RESULTS

Incubation of *C. albicans* cells with I led to a dose-dependent inhibition of [^{14}C]acetate incorporation into ergosterol. Inhibition at lower concentrations of I was accompanied by accumulation of radioactivity in the fraction corresponding to

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Table 1

Effect of I on incorporation of [^{14}C]acetate into non-saponifiable lipid fractions in *C. albicans* cells

Conc. of I (M)	% total radioactivity incorporated by				
	Ergosterol	4α-Methyl-sterol	4,4-Dimethyl-sterol	2,3-Oxido-squalene	Squalene
0 (control)	81.2	6.7	6.3	2.1	3.9
2.4×10^{-7}	75.6	7.0	10.0	2.6	5.0
2.4×10^{-6}	41.3	7.8	36.4	2.7	11.8
2.4×10^{-5}	1.4	5.0	6.8	8.0	78.4
2.4×10^{-4}	0	4.9	1.7	5.4	88.0

Results are mean of 2 separate experiments each with triplicate incubations. Mean incorporation of radioactivity into total non-saponifiable lipids was 151 800 dpm

lanosterol (4,4-dimethylsterols), whereas at 2.4×10^{-5} M and above, radioactivity accumulated in 2,3-oxidosqualene and squalene (table 1). This was confirmed by autoradiography which also revealed accumulation of radioactivity in a more polar fraction, which was not identified (fig.1). A similar pattern of labelling was observed in cells incubated with II, except that no accumulation of activity occurred in the lanosterol fraction. These effects were observed in several independent experiments.

Addition of I to a *C. albicans* cell-free system also caused a dose-dependent inhibition of incorporation of [^{14}C]mevalonate into ergosterol, with accumulation in 2,3-oxidosqualene and squalene (table 2). Inhibitor II showed a similar effect. There was no accumulation of radioactivity in 4,4-dimethylsterol or in the unidentified polar fraction, confirmed by autoradiography of the type shown in fig.1.

Compounds I and II (up to 2.4×10^{-4} M) had no significant effect on incorporation of either acetate or mevalonate into the total non-saponifiable lipids in *C. albicans*. There was thus no evidence for inhibition of steps earlier than squalene epoxidase.

Table 3 summarizes the inhibitory effects of I and II on the microsomal squalene epoxidase and cyclase enzymes. Both compounds inhibited the cyclase enzymes from rat liver and *C. albicans*, as expected from existing reports [1–3]. Compound I was also an effective inhibitor of squalene epoxidase, the rat liver epoxidase being the most sensitive of all the test systems employed. Although this latter inhibition was quantitatively reproducible, Lineweaver-Burk plots were variable between experiments and failed to show a pattern typical of either competitive or non-competitive inhibition.

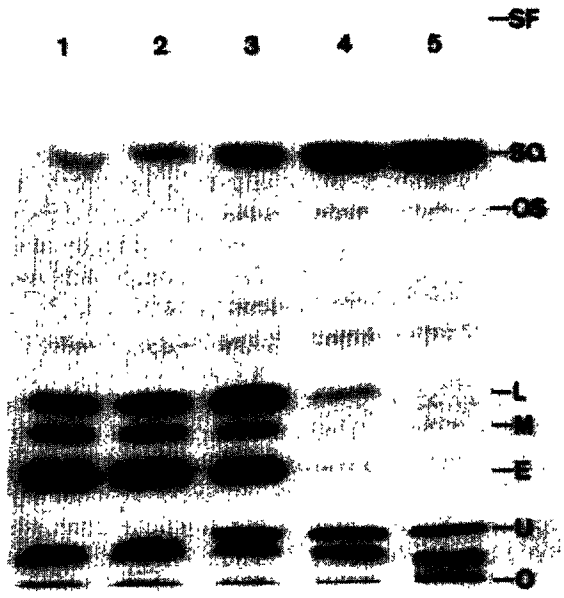

Fig.1. Autoradiograph of TLC separation of labelled non-saponifiable lipids from *C. albicans* cells incubated with [^{14}C]acetate in the presence of different concentrations of I. Concentration of I is: (1) control; (2) 2.4×10^{-7} M; (3) 2.4×10^{-6} M; (4) 2.4×10^{-5} M; (5) 2.4×10^{-4} M. SF, solvent front; SQ, squalene; OS, 2,3-oxidosqualene; L, lanosterol; M, 4α-methylsterols; E, ergosterol; U, unidentified; O, origin.

Table 2

Effect of I on incorporation of [^{14}C]mevalonate into non-saponifiable lipid fractions in *C. albicans* cell-free extract

Conc. of I (M)	% total radioactivity incorporated by				
	Ergosterol	4α-Methyl-sterol	4,4-Dimethyl-sterol	2,3-Oxido-squalene	Squalene
0 (control)	7.5	5.6	6.9	7.8	72.4
2.4×10^{-7}	7.2	5.4	7.1	8.8	71.7
2.4×10^{-6}	5.4	5.2	6.5	9.5	73.6
2.4×10^{-5}	1.3	4.0	3.5	12.1	79.1
2.4×10^{-4}	0	3.7	1.3	6.5	88.6

Results are mean of 2 separate experiments each with triplicate incubations. Mean incorporation of radioactivity into total non-saponifiable lipids was 158 800 dpm

Table 3

Inhibitory effects of I and II on sterol biosynthesis enzymes in *C. albicans* and rat liver

	I_{50} values (μM)	
	I	II
C. albicans epoxidase	32.9	63.0
Rat liver epoxidase	2.4	90.0
C. albicans cyclase	6.5	13.3
Rat liver cyclase	32.2	5.8
C. albicans whole cells	2.5	20.7
C. albicans cell-free	6.6	3.4

Squalene epoxidase, 2,3-oxidosqualene cyclase and measurement of ergosterol biosynthesis in whole cells and cell-free extracts were performed as outlined in section 2. Values are mean of 2 independent experiments each with triplicate incubations

4. DISCUSSION

The similarity to squalene of I and II would suggest them to be competitive inhibitors of both squalene epoxidase and cyclase. Duriatti et al. [3] observed non-competitive kinetics for the inhibition of the cyclase by I, this unexpected result being accounted for by the limitations imposed by a highly lipophilic inhibitor and substrate. This was probably the reason for our failure to obtain satisfactory kinetic plots for the squalene epoxidase inhibition in rat liver microsomes.

Our results show direct inhibition of both squalene epoxidase and cyclase by I in *C. albicans* microsomes. In whole cells of this fungus there was evidence for further, indirect, inhibition of lanosterol demethylation and of some other as yet undefined reaction. It is conceivable that intracellular metabolism of I could lead to formation of other inhibitors, possibly azasteroids which are known to inhibit various stages of ergosterol biosynthesis [10,11].

The lack of specificity and the effects on mammalian enzymes appear to rule out these derivatives as potential antifungal agents, but they may be of interest in the development of hypocholesterolemic agents.

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