ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1985, p. 252–256
0066-4804/85/020252-05$02.00/0

Vol. 27, No. 2

Specific Inhibition of Fungal Sterol Biosynthesis by SF 86-327, a New Allylamine Antimycotic Agent

NEIL S. RYDER

Sandoz Forschungsinstitut, A-1235 Vienna, Austria

Received 4 October 1984/Accepted 26 November 1984

SF 86-327 is a new antimycotic agent of the allylamine type. Its primary action appears to be the inhibition of ergosterol biosynthesis at the point of squalene epoxidation, as was previously found with the related compound naftifine. Biosynthesis was measured by incorporation of [^{14}C]acetate into sterols in cells of *Candida albicans*, *Candida parapsilosis*, *Torulopsis glabrata*, and the dermatophyte *Trichophyton mentagrophytes*. There was a positive correlation between the SF 86-327 concentrations needed for inhibition of growth and of sterol synthesis in these four fungi. The greater antifungal efficacy of SF 86-327 in comparison with naftifine was also reflected in the relative activities of the two compounds as sterol synthesis inhibitors. Inhibition was maximal at neutral pH. A similar degree of inhibition was found in cell-free extracts when [^{14}C]mevalonate was used as substrate. In all cases, inhibition of sterol synthesis was accompanied by a parallel accumulation of labeled squalene. SF 86-327 and naftifine had no significant effect on initial enzymes of the ergosterol pathway, measured by incorporation of [^{14}C]acetyl coenzyme A, or on steps distal to squalene epoxidation, measured by conversion of labeled squalene 2,3-epoxide or lanosterol. Both allylamines were highly selective for fungal, as opposed to mammalian, sterol biosynthesis. SF 86-327 caused slight inhibition of squalene epoxidation in a rat liver cell-free system, but at concentrations three to four orders of magnitude greater than those required for inhibition of the fungal pathway.

SF 86-327 is a new antimycotic agent of the allylamine class. It has in vitro activity against a wide range of pathogenic fungi and is active in vivo after oral or topical application (16). An earlier allylamine antimycotic agent, naftifine (7, 15), has been previously shown to inhibit sterol biosynthesis in *Candida albicans* (18, 19) and some other fungi (14). This inhibition, which occurs at the point of squalene epoxidation, is sufficient to account for the activity of naftifine against *C. albicans* (18). SF 86-327 is a much more potent antifungal agent and it was therefore of great interest to determine the biochemical basis for this activity. The present report describes the qualitative and quantitative effects of SF 86-327 on sterol synthesis in four fungi with different susceptibilities to this compound. In addition, the selectivity of action of both naftifine and SF 86-327 was investigated, since squalene epoxidation is also an essential step in mammalian cholesterol biosynthesis.

(This work was presented in part at the 13th International Congress of Chemotherapy, 28 August to 2 September 1983 [N. S. Ryder, Proc. Int. Congr. Chemother. 13th, Vienna, Austria, p. 4.8/4–4.8/6, 1983] and at the FEMS Conference on the Development of Antifungal Agents, Madrid, Spain, 6 July 1984 [N. S. Ryder, FEMS Conf. Dev. Antifungal Agents, [17a]. p. 133].)

MATERIALS AND METHODS

Fungal cultures. The strains used were *C. albicans* 63 (a clinical isolate), *Candida parapsilosis* ATCC 46589, *Trichophyton mentagrophytes* CBS 56066, and *Torulopsis glabrata* ATCC 15545. Cultures were grown and maintained as described previously (7, 18). *T. mentagrophytes* was grown in Sabouraud Dextrose Broth (E. Merck AG, Darmstadt, Germany) that was adjusted to pH 6.5, inoculated with 10^5 CFU/ml, and incubated for 90 h with shaking at 30°C. MICs were determined by the serial dilution test (7) in Sabouraud medium buffered at pH 6.5 with 0.02 M Britton-Robinson buffer (12). Unbuffered Sabouraud medium (pH 6.5) was used for *T. mentagrophytes*, which did not acidify the unbuffered medium and did not grow well in the buffered medium. Readings were taken after 5 days of incubation with the yeasts and after 7 days of incubation with *T. mentagrophytes*.

Radiolabeled compounds. [U-^{14}C]acetate, [2-^{14}C]mevalonic acid lactone, [1,2-^{3}H]cholesterol, and [1-^{14}C]acetylcoenzyme A were obtained from Amersham International, Amersham, England. Labeled squalene 2,3-epoxide was prepared biosynthetically in a rat liver cell-free system by using [2-^{14}C]mevalonate (specific activity, 53 Ci/mol) as described below. Incubations contained 1 mM AMO-1618 (Calbiochem AG, Lucerne, Switzerland) to block squalene epoxidocyclase (4). The [^{14}C]squalene epoxide was isolated by thin-layer chromatography (18) and eluted in chloroform-methanol (3:1, vol/vol). Crude preparations of [^{14}C]lanosterol were similarly made with the *C. albicans* cell-free system described below. Cell extracts which had low lanosterol demethylation activity, and therefore accumulated the desired product, were selected.

Sterol biosynthesis in fungal cells. Incubation of cells with [^{14}C]acetate was performed as described previously (18), with slight modification of the subsequent procedure for isolation of sterols. After incubation, yeast cells were pelleted in glass tubes and treated with 5 ml of 0.1 M HCl for 15 min in a water bath at 90°C. This improved the efficiency of extraction (8) without affecting the resulting sterol composition. *T. mentagrophytes* cells were collected by filtration on glass fiber filters (GFA, 25 mm; Whatman, Inc., Clifton, N.J.). Saponification and extraction of the nonsaponifiable lipids (NSLs) were carried out in the glass tubes. The components of the NSLs were separated by thin-layer chromatography as described previously (18), except plates with a sample-concentrating zone (Merck no. 5583) were used.

Fungal cell-free sterol biosynthesis. Incorporation of [^{14}C]mevalonate into sterols by *Candida* cell-free extracts

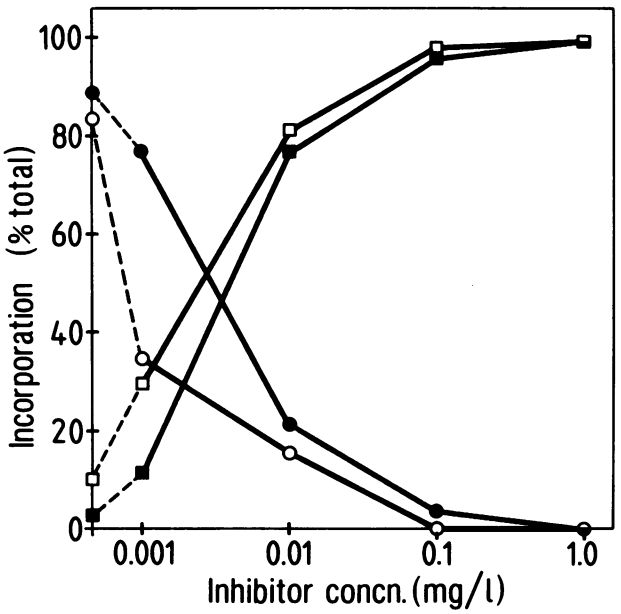

FIG. 1. Effect of SF 86-327 and naftifine on incorporation of [^{14}C]acetate into ergosterol and squalene in *T. mentagrophytes* cells. Points are the means of triplicate incubations. Shown are ergosterol (○) and squalene (□) in the presence of SF 86-327 and ergosterol (●) and squalene (■) in the presence of naftifine.

was carried out as described previously (18), except the incubations contained in addition 0.1 mM flavin adenine dinucleotide. The same conditions were used for incorporation of [^{14}C]acetyl-coenzyme A (specific activity, 5 Ci/mol; final concentration, 0.2 μCi/ml). With labeled squalene epoxide or lanosterol as substrate, incubations consisted of 5 μl of ethanol (containing test compound), 455 μl of cell-free extract, 30 μl of cofactor mixture (as described above), and 10 μl of substrate solution (2×10^4 dpm in acetone containing 1% Tween 80). In all cases, separation of the NSL components was carried out as described previously (18).

Sterol biosynthesis in rat liver extracts. The method for sterol biosynthesis was modified from that of Popjak (17). Livers were removed from freshly killed, male Lewis rats (12 to 16 weeks old) and chilled in ice. Livers were rinsed in 0.1 M phosphate buffer (pH 7.4) containing 30 mM nicotinamide, 2 mM $MnCl_2$, 3 mM $MgCl_2$, and 0.5 mM dithiothreitol; trimmed free of extraneous tissue; cut into small pieces; and thoroughly washed in buffer. Portions of liver (20 g, wet weight) were homogenized with 25 ml of buffer for 10 s in an Ultraturrax homogenizer at the 70% setting. The pooled homogenates were centrifuged at 3,000 × *g* for 10 min, and the supernatant was collected through a fine sieve. The supernatant was recentrifuged at 10,000 × *g* for 10 min, and the floating lipid was removed. The supernatant was stored in plastic ampoules in liquid nitrogen and used as the cell-free extract. Sterol biosynthesis with [^{14}C]mevalonate as substrate was assayed in the same way as described above for *C. albicans*.

General methods. Protein content of cell-free extracts was assayed with the Bio-Rad reagent (Bio-Rad Laboratories, Richmond, Calif.) with bovine gamma globulin as standard. In radiolabeling assays, test compounds were added to incubation mixtures as a solution in ethanol (fungal systems) or dimethyl sulfoxide (rat liver). In all cases the final concentration of solvent was 1% and an equal amount was added to controls. All the test systems employed were adjusted so that incorporation into sterols was linear with respect to time and to cell or protein concentration. In all experiments, [^{3}H]cholesterol was added as an internal standard to correct for losses during handling, and ^{14}C-labeled products were then measured by dual-label scintillation counting (18). All experiments were performed with at least three replicate incubations per treatment and were repeated to obtain consistent results.

RESULTS

Effects on fungal sterol biosynthesis. Preliminary experiments with *C. albicans* indicated that SF 86-327 inhibits sterol biosynthesis at the point of squalene epoxidation, as has been previously demonstrated with naftifine (18). This effect was then investigated in detail in four representative fungal strains chosen for their various degrees of susceptibility to these compounds. In cells of the dermatophyte *T. mentagrophytes*, ergosterol biosynthesis was highly sensitive to inhibition by both naftifine and SF 86-327 (Fig. 1). Both compounds caused a dose-dependent inhibition of [^{14}C]acetate incorporation into ergosterol, with a parallel accumulation of radiolabeled squalene. SF 86-327 was the more effective inhibitor (Fig. 1). A qualitatively similar pattern of inhibition was observed in cells of *C. albicans*, *C. parapsilosis*, and *T. glabrata*.

To determine whether observed differences in the sensitivity of sterol synthesis in whole cells of different fungi were due to variation in uptake of the compounds, I investigated the effects on cell-free sterol biosynthesis. In this case also, SF 86-327 powerfully inhibited incorporation of the substrate [^{14}C]mevalonate into ergosterol. Squalene was the only sterol precursor which accumulated. Table 1 shows this to be true for *C. parapsilosis*, and a similar effect was found in the other two yeasts tested. Squalene was the sole radiolabeled product detectable by autoradiography (performed as described previously [18]) in all incubations treated with SF 86-327 at a concentration of 1 mg/liter or higher. In cell-free extracts of *T. mentagrophytes*, biosynthesis proceeded only as far as squalene, and it was therefore not possible to test the allylamines in this system.

Curves of the type shown in Fig. 1 were used to obtain quantitative data from a large number of radiolabeling experiments. These data are shown in Table 2 in comparison with MICs obtained in a medium similarly buffered at pH 6.5. There was a positive correlation between inhibition of growth and of sterol biosynthesis, the dermatophyte being exceptionally sensitive in both cases. On the other hand, the large differences in MICs among the three yeasts are not fully reflected in the values for inhibition of sterol biosynthesis. A similar degree of correlation was found with naftifine when the same four strains of fungi were used.

TABLE 1. Effect of SF 86-327 on incorporation of [^{14}C]mevalonate into sterols and squalene in *C. parapsilosis* cell-free extract

SF 86-327 concn (mg/liter)	% Total radioactivity incorporated by:			
	Ergosterol	4α-Methyl-sterol	4,4-Dimethyl-sterol	Squalene
0 (Control)	33.2	6.3	31.6	28.9
0.001	30.5	5.9	31.8	31.9
0.01	10.7	1.5	5.4	82.3
0.1	0.5	0.8	0.4	98.4
1.0	0.1	0.7	0.2	98.9

In all cases tested, sensitivity to inhibition by SF 86-327 was similar in whole cells and in cell-free extracts (Table 2), as previously reported for naftifine in *C. albicans* (18). Thus, cell penetration does not seem to be a factor limiting the activity of these compounds.

Effect on squalene biosynthesis. It was earlier reported (14, 19) that treatment of cells with naftifine led to increased incorporation of [^{14}C]acetate into the total NSLs. This effect was further investigated with both naftifine and SF 86-327 (Table 3). The stimulatory effect was concentration dependent and variable, depending on the combination of inhibitor and fungal strain. No effects were observed on the cell-free incorporation of [^{14}C]mevalonate into total NSLs, suggesting a possible stimulatory action on earlier enzymes of the pathway. This possibility was tested by measuring the incorporation of [^{14}C]acetyl-coenzyme A into NSLs in a *C. albicans* cell-free system. The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor compactin (5) caused 92% inhibition at 0.5 mg/liter in this assay system. Naftifine or SF 86-327 at concentrations of up to 10 mg/liter had no significant effect, indicating the absence of any direct influence on the initial enzymes of the sterol pathway.

Effect of pH. The activity of SF 86-327 and naftifine as inhibitors of sterol biosynthesis was measured in *C. albicans* cells buffered at different pH values (Fig. 2). In both cases, inhibition was pH sensitive and maximal at neutral pH. Similar pH sensitivity was found in *C. parapsilosis* and *T. mentagrophytes*. These results are in agreement with the previously noted pH dependence of growth inhibition by naftifine (7).

Effects on later stages of ergosterol biosynthesis. Because of the strong inhibition of squalene epoxidation by the allylamines, effects on subsequent steps in the pathway would be difficult to detect in the test systems described. Earlier work (18, 19) has suggested the possible existence of a second inhibitory action of naftifine at the level of the 4α-methylsterols. Effects on processes distal to squalene epoxidation were therefore measured directly by use of [^{14}C]squalene epoxide and [^{14}C]lanosterol as substrates in cell-free test systems. Positive controls were provided by AMO-1618, which inhibits squalene epoxidocyclase (4), and ketoconazole, which inhibits lanosterol 14α-demethylation (20, 21). Cell-free extracts of *C. albicans* and *C. parapsilosis* converted the [^{14}C]lanosterol to 4-desmethylsterols (ergosterol), the process being linear with time for at least 4 h. Ketoconazole (1 mg/liter) caused 94% inhibition of the conversion. Naftifine or SF 86-327 at concentrations of up to 10 mg/liter had no significant effect on the system from either *Candida* strain. At 100 mg/liter, both compounds caused a slight (10 to 20%) inhibition which, at such a high concentration, may be ascribed to nonspecific interactions of the lipophilic compounds with membrane-bound enzymes. *Trichophyton* cell-free extracts did not convert [^{14}C]lanosterol.

Cell-free extracts of *C. albicans* and *C. parapsilosis* cyclized [^{14}C]squalene epoxide to lanosterol, which was then further converted to ergosterol. Naftifine and SF 86-327 at concentrations of up to 100 mg/liter had no effect on this reaction, whereas 1 mM AMO-1618 caused ca. 50% inhibition. In *T. mentagrophytes* cell-free extracts, the conversion proceeded to lanosterol only and was partially inhibited by higher concentrations of the allylamines (Table 4).

Effects on biosynthesis of rat liver cholesterol. The effects on the biosynthesis of rat liver cholesterol were investigated in a cell-free test system very similar to that used for the fungal strains. Within the 2-h assay, 10 to 15% of the [^{14}C]mevalonate was converted into NSL, of which up to 50% was found in the cholesterol fraction. The imidazole

TABLE 2. SF 86-327 concentrations causing inhibition of fungal growth (MICs) and sterol biosynthesis[a]

Fungus	MIC (μg/ml)	System	Inhibitory concn (%)[b]	
			50	95
T. mentagrophytes	0.003	Cells	0.002	0.04
C. parapsilosis	0.4	Cells	0.006	0.3
		Cell-free	0.003	0.1
C. albicans	3.1	Cells	0.008	0.2
		Cell-free	0.008	0.3
T. glabrata	>100	Cells	0.040	0.9
		Cell-free	0.035	1.0

[a] Sterol biosynthesis was measured by incorporation of [^{14}C]acetate in cells and [^{14}C]mevalonate in cell-free extracts.

[b] Results shown are the means of three separate experiments. The standard error was less than 30% of the mean in all cases.

TABLE 3. Effect of SF 86-327 and naftifine on incorporation of [^{14}C]acetate into NSLs in whole cells

Compound and concn (mg/liter)	Mean ± SE (% of control) incorporated into NSL (no. of expts) by:		
	T. mentagrophytes	*C. albicans*	*C. parapsilosis*
SF 86-327			
0.1	124 ± 7 (5)	118 ± 14 (4)	146 ± 14 (4)
1.0	123 ± 3 (5)	109 ± 19 (20)	144 ± 13 (9)
10.0		94 ± 18 (10)	137 ± 16 (4)
Naftifine			
0.1	133 ± 14 (3)	147 ± 22 (3)	140 ± 12 (2)
1.0	117 ± 6 (3)	138 ± 19 (6)	126 ± 20 (4)
10.0		144 ± 16 (13)	94 ± 15 (4)

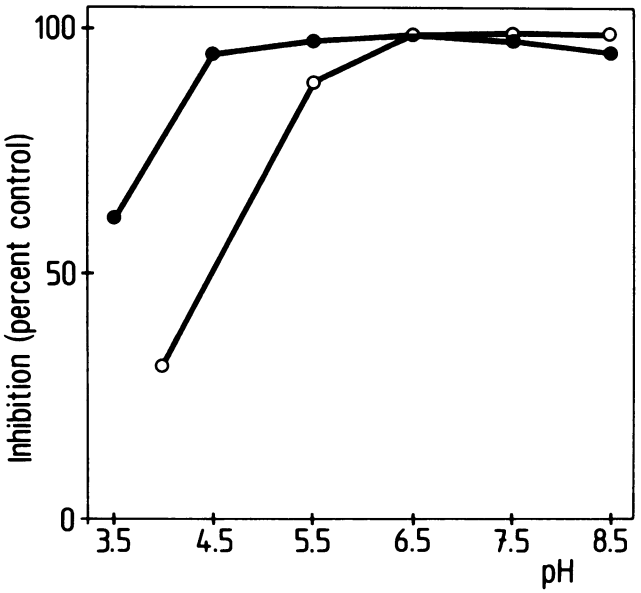

FIG. 2. Effect of pH on sterol synthesis inhibition in *C. albicans* cells by SF 86-327 and naftifine. Sterol synthesis was measured by incorporation of [^{14}C]acetate into ergosterol, and inhibition was expressed as a percentage of the control at each pH value. Symbols: ●, SF 86-327 at 1 mg/liter; ○, naftifine at 10 mg/liter.

TABLE 4. Effect of SF 86-327 and naftifine on conversion of [^{14}C]squalene epoxide to 4,4-dimethylsterol in *T. mentagrophytes* cell-free extracts

Concn (mg/liter)	% Inhibition by[a]:	
	SF 86-327	Naftifine
1	0.0	0.0
10	6.5	0.0
30	12.8	28.2
100	45.0	28.7

[a] Results shown are the means of two separate experiments.

antimycotics ketoconazole and clotrimazole served as positive controls, as this class of compounds has previously been shown to inhibit cholesterol biosynthesis (2, 22). Both imidazoles were effective inhibitors of this system, with concentrations causing 50% inhibition of less than 1 mg/liter (Table 5). In contrast, naftifine had little specific effect on sterol biosynthesis (concentration causing 50% inhibition, >100 mg/liter). SF 86-327 was only weakly inhibitory (Table 5), with a concentration causing 50% inhibition of about 30 mg/liter (mean of three experiments). This degree of inhibition was found to be constant with increasing incubation times of 30 min to 2 h, showing that the low extent of inhibiton was not due to progressive destruction of the SF 86-327 by liver enzymes. The drop in incorporation into cholesterol caused by SF 86-327 was balanced by increased accumulation of squalene and, to a lesser extent, lanosterol (Table 6). The slight increase in labeled lanosterol suggests the occurrence of nonspecific inhibition of later stages of the pathway at very high concentrations of SF 86-327. Clearly, the primary effect of SF 86-327 on cholesterol biosynthesis is inhibition of squalene epoxidation, as in fungi. However, the concentration of SF 86-327 required for inhibition of rat liver cholesterol biosynthesis was three to four orders of magnitude greater than that required for inhibition of fungal ergosterol biosynthesis (see Table 2).

TABLE 5. Effect of antimycotic compounds on incorporation of [2-^{14}C]mevalonate into NSLs and cholesterol in rat liver cell-free extract

Compound and concn (mg/liter)	Incorporation (% of control) into[a]:	
	NSL	Cholesterol[b]
Naftifine		
1	97.8	91.2
10	90.1	88.3
100	54.6	79.8
SF 86-327		
1	107.2	98.7
10	102.8	64.5
100	99.0	34.4
Ketoconazole		
0.1	99.4	91.7
1	107.6	39.9
10	114.1	12.1
Clotrimazole		
0.1	101.4	103.0
1	114.6	29.1
10	115.9	16.4

[a] Results shown are the means of two separate experiments, each performed in triplicate.

[b] Incorporation into cholesterol was calculated as a proportion of the total NSL and then expressed as a percentage of the appropriate control value.

DISCUSSION

The results indicate that the primary mode of action of SF 86-327 involves inhibition of ergosterol biosynthesis at the point of squalene epoxidation, as is true of the related compound naftifine (14, 18, 19). This conclusion is supported by the correlation between inhibition of growth and of sterol biosynthesis in the four fungi used in this study. Similarly, the superior efficacy of SF 86-327 as an antifungal agent is reflected in its greater potency as a sterol synthesis inhibitor in comparison with naftifine. In cells of the three yeasts, the MIC of SF 86-327 was in each case sufficient for total inhibition of sterol biosynthesis. This was not the case, however, in the dermatophyte *T. mentagrophytes*, for which the MIC was considerably lower than that required for 95% inhibition of sterol biosynthesis (Table 2). At the very low concentrations involved with this organism, the results may be influenced by the degree of nonspecific binding of inhibitor to the cells. Cell concentration was necessarily much higher in the labeling experiments than in the MIC determinations.

Differences in sensitivity of the sterol synthesis pathway to inhibition by the allylamines clearly influence the susceptibility of the different fungi to growth inhibition. These differences, however, do not appear to be sufficient to explain the widely differing MICs for the three yeasts. The close agreement between inhibitory concentrations in the whole cell and in cell-free test systems suggests that penetration of the cell envelope is not a limiting factor. It would therefore seem likely that the fungi differ in their inherent susceptibilities to blockade of sterol synthesis or accumulation of intracellular squalene or both. For example, fermentative yeasts such as *T. glabrata* or *Saccharomyces cerevisiae* are adapted to withstand conditions of low ergosterol content and high squalene levels during anaerobic growth (3, 6, 10) and would thus not be expected to be highly susceptible to the effects of the allylamines.

The inhibitory effects of SF 86-327 and naftifine were found to be pH dependent, with maximal activity at around neutral pH, which correlates with the pH dependency of growth inhibition (7, 18). A similar effect of pH has been reported with the imidazole antimycotics (1, 12), underlining the need to use buffered media for comparative in vitro testing, since most fungi tend to acidify the commonly used growth media.

The present results show that both SF 86-327 and naftifine specifically inhibit squalene epoxidation only and have no significant effect on other stages of the sterol synthesis pathway. In particular, the allylamines have no effect on

TABLE 6. Effect of SF 86-327 and ketoconazole on incorporation of [2-^{14}C]mevalonate into NSL fractions by rat liver cell-free extract

Compound and concn (mg/liter)	% Total radioactivity incorporated by[a]:			
	Cholesterol	4α-Methyl-sterol	4,4-Dimethyl-sterol	Squaline
Control	42.5	18.5	19.4	17.0
SF 86 327				
1.0	49.8	17.1	16.7	16.5
10	41.3	20.5	20.6	17.6
100	26.5	14.5	27.1	31.8
Ketoconazole (1.0)	13.0	10.2	66.4	10.4

[a] Results shown are the means of five replicate incubations.

lanosterol 14α-demethylation, the cytochrome P-450-mediated step inhibited by azole antifungal agents (20, 21). A slight inhibition of steps distal to squalene epoxidation occurred only at substance concentrations too high to be of therapeutic relevance and was probably due to nonspecific lipophilic interactions with the membrane-bound enzymes. In addition, the inhibition of sterol biosynthesis measured in *C. albicans* (Table 2) can be fully accounted for by the recently described inhibition of squalene epoxidase (16).

Since SF 86-327 is under development as an orally administered antimycotic, it is important to establish the extent of its interactions with mammalian cholesterol biosynthesis, which also involves squalene epoxidation. The results presented here demonstrate that SF 86-327 has a highly selective action against fungal sterol synthesis, the rat liver system differing in sensitivity by three to four orders of magnitude. Squalene is a normal constituent of bodily tissues and, in view of the contribution of dietary sterol to the overall maintenance of cholesterol levels in the body, a partial inhibition of squalene epoxidase would be of little physiological significance. However, the level of SF 86-327 required for significant inhibition of mammalian sterol synthesis is unlikely to be attained in vivo. Furthermore, squalene epoxidase, the primary target of the allylamines, is not a cytochrome P-450 enzyme (13), and so there is no likelihood of inhibiting this class of enzyme during therapy, as has been reported to occur with imidazole antimycotics (9, 11).

ACKNOWLEDGMENTS

I thank M.-C. Dupont, I. Frank, and M. Jahoda for technical assistance.

LITERATURE CITED

1. **Beggs, W. H.** 1982. Influence of pH on ketoconazole activity against *Candida parapsilosis* and *Candida albicans*. Res. Commun. Chem. Pathol. Pharmacol. **37**:495–498.
2. **Buttke, T. M., and S. W. Chapman.** 1983. Inhibition by ketoconazole of mitogen-induced DNA synthesis and cholesterol biosynthesis in lymphocytes. Antimicrob. Agents Chemother. **24**:478–485.
3. **Buttke, T. M., and A. L. Pyle.** 1982. Effects of unsaturated fatty acid deprivation on neutral lipid synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **152**:747–756.
4. **Douglas, T. J., and L. G. Paleg.** 1978. AMO-1618 and sterol biosynthesis in tissues and sub-cellular fractions of tobacco seedlings. Phytochemistry **17**:705–712.
5. **Endo, A., M. Kuroda, and K. Tanzawa.** 1976. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity. FEBS Lett. **72**:323–326.
6. **Fryberg, M., A. C. Oehlschlager, and A. M. Unrau.** 1973. Biosynthesis of ergosterol in yeast. Evidence for multiple pathways. J. Am. Chem. Soc. **95**:5747–5757.
7. **Georgopoulos, A., G. Petranyi, H. Mieth, and J. Drews.** 1981. In vitro activity of naftifine, a new antifungal agent. Antimicrob. Agents Chemother. **19**:386–389.
8. **Gonzales, R. A., and L. W. Parks.** 1977. Acid-labilization of sterols for extraction from yeast. Biochim. Biophys. Acta **489**:507–509.
9. **Grosso, D. S., T. W. Boyden, R. W. Pamenter, D. G. Johnson, D. A. Stevens, and J. N. Galgiani.** 1983. Ketoconazole inhibition of testicular secretion of testosterone and displacement of steroid hormones from serum transport proteins. Antimicrob. Agents Chemother. **23**:207–212.
10. **Klein, H. P.** 1955. Synthesis of lipids in resting cells of *Saccharomyces cerevisiae*. J. Bacteriol. **69**:620–627.
11. **Loose, D. S., P. B. Kan, M. A. Hirst, R. A. Marcus, and D. Feldman.** 1983. Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P-450-dependent enzymes. J. Clin. Invest. **71**:1495–1499.
12. **Minagawa, H., K. Kitaura, and N. Nakamizo.** 1983. Effects of pH on the activity of ketoconazole against *Candida albicans*. Antimicrob. Agents Chemother. **23**:105–107.
13. **Ono, T., K. Nakazono, and M. Kosaka.** 1982. Purification and partial characterization of squalene epoxidase from rat liver microsomes. Biochim. Biophys. Acta **709**:84–90.
14. **Paltauf, G., G. Daum, G. Zuder, G. Högenauer, G. Schulz, and G. Seidl.** 1982. Squalene and ergosterol biosynthesis in fungi treated with naftifine, a new antimycotic agent. Biochim. Biophys. Acta **712**:268–273.
15. **Petranyi, G., A. Georgopoulos, and H. Mieth.** 1981. In vivo antimycotic activity of naftifine. Antimicrob. Agents Chemother. **19**:390–392.
16. **Petranyi, G., N. S. Ryder, and A. Stütz.** 1984. Allylamine derivatives: new class of synthetic antifungal agents inhibiting fungal squalene epoxidase. Science **224**:1239–1241.
17. **Popjak, G.** 1969. Enzymes of sterol biosynthesis in liver and intermediates of sterol biosynthesis. Methods Enzymol. **25**:393–454.

17a. **Ryder, N. S.** 1984. Selective inhibition of squalene epoxidation by allylamine antimycotic agents, p. 313–321. *In* C. Nombela (ed.), Microbial cell, wall synthesis and autolysis. Elsevier Science Publishers, Amsterdam.

18. **Ryder, N. S., G. Seidl, and P. F. Troke.** 1984. Effect of the antimycotic drug naftifine on growth of and sterol biosynthesis in *Candida albicans*. Antimicrob. Agents Chemother. **25**:483–487.
19. **Ryder, N. S., and P. F. Troke.** 1981. The activity of naftifine as a sterol synthesis inhibitor in *Candida albicans*, p. 1016–1017. *In* P. Periti and G. G. Grassi (ed.), Current chemotherapy and immunotherapy, vol. 2. American Society for Microbiology, Washington, D.C.
20. **Van den Bossche, H., G. Willemsens, W. Cools, F. Cornelissen, W. F. Lauwers, and J. M. Van Cutsem.** 1980. In vitro and in vivo effects of the antimycotic drug ketoconazole on sterol synthesis. Antimicrob. Agents Chemother. **17**:922–928.
21. **Van den Bossche, H., G. Willemsen, W. Cools, P. Marichal, and W. Lauwers.** 1983. Hypothesis on the molecular basis of the antifungal activity of *N*-substituted imidazoles and triazoles. Biochem. Soc. Trans. **11**:665–667.
22. **Willemsens, G., W. Cools, and H. Van den Bossche.** 1980. Effects of miconazole and ketoconazole on sterol synthesis in a subcellular fraction of yeast and mammalian cells, p. 691–694. *In* H. Van den Bossche (ed.), The host invader interplay. Elsevier/North-Holland Biomedical Press, Amsterdam.