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SHORT COMMUNICATION

A NOVEL LANOSTEROL ISOMER PRODUCED IN RESPONSE TO AZOLE ANTIFUNGALS

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Summary—Gas chromatography–mass spectrometry has revealed the existence of a novel lanosterol-like sterol which is produced by fungi in response to treatment with azole drugs. The significance of this finding may be related to the changes in fungal sterol synthesis as a consequence to prolonged exposure to azoles and consequent development of resistance to these agents.

INTRODUCTION

Several species of fungi following treatment with azole antifungal agents have been observed to produce a sterol closely related to, but differentiated from lanosterol. The mass spectra of the tri-methylsilyl (TMS) and the tert-butyldimethylsilyl (*t*BDMS) ethers were qualitatively similar showing differences principally in the intensities of the $[M-15]^+$ and $[M-57]^+$ ions. The *t*BDMS derivative of the new sterol was clearly separated from that of authentic lanosterol.

EXPERIMENTAL

Strains

The source of the fungal strains has been described elsewhere [1]. The filamentous fungi used were *Trichophyton mentagrophytes* (two strains), *T. rubrum* (two), *Microsporum gypseum* (one), *Epidermophyton floccosum* (one), *Hendersonula toruloidea* (three strains each of forms 1, 2 and 3) and *Scytalidium hyalinum* (two). Isolates of *Candida albicans* included five azole sensitive strains lyophilized in 1960 by Professor W. C. Noble, Institute of Dermatology, St Thomas' Hospital, London; and four strains from the Mycological Reference Laboratory, Colindale, London, NCPF 3153 (azole sensitive), NCPF 3302 and 3303 (azole resistant, [2]) and NCPF 3310 (azole resistant, [3]).

Growth

All fungi were grown to log phase in Sabouraud's dextrose broth (40 g/l dextrose, 10 g/l bactopeptone) on an orbital incubator. The filamentous fungi were grown at 30°C, challenged with azole and reincubated for a further 24 h before the mycelia were collected. The yeasts were grown at 37°C and incubated for up to 7 h after drug exposure. The concentrations of drug were half the MIC determined for each isolate under the stated conditions. Miconazole was used, although ketoconazole and itraconazole were found to give similar results.

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Sterol extraction and analysis

Sterols were extracted by alkaline saponification and derivatized to form either the *t*BDMS or the TMS ethers [1]. These were analysed on a Hewlett–Packard 5890 gas chromatograph using 12 m × 0.32 mm SE30 coated quartz capillary columns coupled to the source of a V. G. Analytical Ltd 70 VSEQ mass spectrometer. Mass spectra and accurate mass measurements were performed using electron impact ionisation.

RESULTS AND DISCUSSION

The present study arose from an investigation of the changes in sterol biosynthesis in fungi as a function of their reaction to exposure to antifungal agents and an attempt to correlate these with development of antifungal resistance. Following drug exposure, a sterol was detected that differed in its mass spectrum from authentic lanosterol in the intensity of the $[M-15]^+$ ion as the TMS ether, and the $[M-57]^+$ ion as the *t*BDMS ether. The latter derivative also eluted from the GC column significantly earlier than the authentic lanosterol. Accurate mass measurements of the four most intense high-mass fragment ions seen in the *t*BDMS spectra could be assigned identical molecular formulae for both the fungal sterol and for lanosterol (Table 1). The error values between the measured and the calculated masses were within acceptable limits. These results suggest that the fungal compound is an isomer of lanosterol.

This lanosterol isomer accumulated in many of the filamentous fungi after azole treatment and in two *C. albicans* strains, but was present in only small amounts prior to treatment (Table 2). The production of this sterol did not correlate with azole sensitivity as it was not detected in all of the sensitive strains and was present in *C. albicans* NCPF 3310, although this strain has been well documented as having an unusual mode of resistance [4, 5]. Neither did it appear to influence the structure of sterols produced at later stages in sterol biosynthesis as no differences were observed in the mass spectra or retention times of sterols from treated and untreated cultures. The detection of this isomer further indicates the diversity of biosynthetic processes with which fungi can produce sterols.

Table 1. Accurate mass measurements of ions in the spectra of the *t*BDMS ethers of lanosterol and the lanosterol-like compound

Mass found			Max error
Lanosterol	Lanosterol-like	Formula	(mDa)
540.4737	540.4693	$C_{36}H_{64}OSi$	3.3
525.4484	525.4469	$C_{35}H_{61}OSi$	2.2
483.3957	483.4003	$C_{32}H_{55}OSi$	6.4
393.3524	393.3510	$C_{29}H_{45}$	1.1

Table 2. Production of lanosterol and lanosterol-like sterol by fungi in response to exposure to imidazole

	Lanosterol		Lanosterol-like	
Species	Before	After	Before	After
T. mentagrophytes	+	−	−	+ +
T. rubrum	+	−	−	+ +
M. gypseum	+	−	+	+ + +
E. floccosum	−	−	−	+ +
H. toruloidea: Form 1	+ +	+ +	+ + (1/3) − (2/3)	+ +
Form 2	+ +	+ +	−	+ (1/3) − (2/3)
Form 3	+	+	− (2/3) + (1/3)	+ + (1/3) + (2/3)
S. hyalinum	+	+	−	+ +
C. albicans: NCPF 3310	+	+	+	+
NCPF 3302	−	+ +	−	−
NCPF 3303	+ +	+ +	−	−
NCPF 3153	+	+ +	−	+ + +
1960's strains	+ (1/5) + + (4/5)	+ (4/5) + + + (1/5)	−	+ (1/5) − (4/5)

(1/3) refers to the number of strains producing the sterol. — = not detected (> 0.02%); + = 0.02–0.9%; + + = 1–9.9%; + + + = ⩾ 10% of total sterol content.

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