Biosynthetic Implications of the Sterol Content of Ergosterol-deficient Mutants of Yeast

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Summary Four nystatin-resistant mutants of *Saccharomyces cerevisiae*, have been shown, by an analysis of the sterols present, to contain metabolic blocks (in normal ergosterol biosynthesis) for C-24 transmethylation, the $\Delta^8 \rightarrow \Delta^7$-isomerase, the 5,6-dehydrogenase, and the 22,23-dehydrogenase respectively; the previously unknown sterols, cholesta-5,7,22,24-tetraen-3β-ol, ergosta-8,22,24(28)-trien-3β-ol, and ergosta-8,14,24(28)trien-3β-ol were isolated and characterised.

WE[1] and others[2] have previously shown that the terminal stages of ergosterol biosynthesis in *Saccharomyces cerevisiae* consist of a multiplicity of pathways derived from five basic transformations. It was not known whether these transformations are each mediated by single, nonspecific enzymes or by many specific ones. One of us[3] has described the isolation of a number of ergosterol-deficient mutants of yeast by selection of strains from a nystatin-containing growth medium. Accordingly we undertook a sterol analysis of these mutants (designated pol 1, 2, 3, and 5) in order to determine the position of the metabolic block, and the completeness of this block.

The mutants and the (precursor) wild type, were grown aerobically to stationary phase (24 h), harvested, saponified, and the non-saponifiable fraction benzoylated. Preparative t.l.c. on silica afforded the mixed 4-demethyl sterol benzoates which were assayed spectroscopically. Further separation of the mixture was achieved by repeated silver nitrate–silica gel t.l.c. which resolved all components except for sterols differing only in the presence of double bonds at 7 or 8(9). These were, however, readily distinguished by n.m.r. spectroscopy. The results are given in the Table.

TABLE
Major sterols of pol mutants of *Saccharomyces cerevisiae*

Mutant	Sterol	Enzyme block
Wild Type A184 D	Zymosterol Ergosterol	—
Pol 1	Zymosterol Cholesta-5,7,24-trien-3β-ol Cholesta-5,7,22,24-tetraen-3β-ol[a]	C-24 Methyl transferase
Pol 2	Ergost-8-en-3β-ol Ergosta-8,22-dien-3β-ol Ergosta-5,8,22-trien-3β-ol Fecosterol	$\Delta^8 \rightarrow \Delta^7$ Isomerase
Pol 3	Ergosta-7,22-dien-3β-ol Ergosta-8,22-dien-3β-ol Ergosta-7,22,24(28)-trien-3β-ol Ergosta-8,22,24(28)-trien-3β-ol[a] Episterol Fecosterol	[b] [b] 5,6-Dehydrogenase [b]
Pol 5	Episterol Ergosta-5,7-dien-3β-ol Ergosta-5,7,24(28)-trien-3β-ol Ergosta-8,14,24(28)-trien-3β-ol[a]	22,23-Dehydrogenase

[a] All new compounds were fully characterised by microanalytical and/or spectroscopic methods.
[b] Δ^7, Δ^8 pairs were not separated.

only of the mixture, but further confirmation of the implied metabolic blocks was obtained as follows.

For pol. 1, which apparently lacked methyl transferase activity, a mass spectrum of the total 4-demethyl fraction showed no ions corresponding to C-28 sterols. Pol 2 and pol 3, which apparently lacked respectively the $\Delta^8 \rightarrow \Delta^7$ isomerase and 5,6-dehydrogenase activities had no ring B diene chromophore in the crude sterol mixture and pol 5, which apparently lacked the 22,23-dehydrogenase similarly showed no side-chain diene absorption in the u.v. region.

The pol strains have been shown to be single mutants.[3,4] The effect of each mutation is a complete loss of activity of one enzymic process in the terminal stages of ergosterol formation. It follows that only one enzyme (or enzyme complex) is involved for each transformation, whatever the substrate. The sterol spectra of the mutants also show that the terminal transformations are performed independently, within the logical constraints of the system. Since the remainder of the enzymes are unaffected by the mutation, the formation of the sterols reported here must be a potentially normal activity and the schemes for ergosterol biosynthesis proposed previously[1,2] must be expanded. Because of the complexity of the scheme that now emerges, we consider that the biosynthesis, at least at the post-zymosterol stage, is best described as simply a combination of the four basic processes in the Table with the addition of a 24(28)-hydrogenase.

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