NOVEL STEROLS IN ERGOSTEROL DEFICIENT YEAST MUTANTS*

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Received June 4,1974

Summary. Sterols of nystatin resistant and ergosterol requiring mutants, and of the wild type parent of Saccharomyces cerevisiae were separated by a newly developed procedure and were identified. The mutants contained larger amounts of lanosterol (I) than the wild type, as well as 4, 14-dimethylcholesta-8, 24-dien-3β-ol (II), 4, 14-dimethylergosta-8, 24(28)-dien-3β-ol (III), and 14-methyl-ergosta-8, 24(28)-dien-3β-ol (IV), which were not hitherto found in yeast. These results indicated a block in removal of the methyl group at C-14 of lanosterol.

Sterol deficient mutants of yeast have been investigated recently in an effort to clarify ergosterol formation and its regulation (1, 2). The mutants isolated in our laboratory required ergosterol as sole lipid supplement. They also required methionine (or homocysteine) for growth, and were petite (respiratory deficient) owing to a mutation in porphyrin biosynthesis (2, 3). The homocysteine requirement may be due to lack of porphyrin for sulfite reductase. Cells grown on heme were respiratory competent and did not require methionine. Revertants of these strains were nystatin resistant, indicating that a mutation in sterol biosynthesis, had occurred. We have developed new methods for separating yeast sterols, and have analyzed the sterol composition of wild type, nystatin resistant, and ergosterol requiring strains. The results showed accumulation of novel sterols not hitherto found in yeast.

* This work was supported by grants from the American Heart Association, the National Institutes of Health of the U. S. Public Health Service, and the National Science Foundation.

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Materials and Methods. Strains D-587-4B (αhis 1-1), Nys 1, and Erg2 of Saccharomyces cerevisiae (2) were grown and harvested as previously described (2). Sterols were initially separated by thin layer chromatography on $AgNO_3$ impregnated silica. Further separation was acheived on free sterols by liquid chromatography (Waters Associates model LC202), and on their trimethylsilyl ethers by gas chromatography (Hewlett-Packard model 5711A). Pure lanosterol was similarly prepared from the commercial product. Detailed procedures will be described elsewhere. Mass spectra were obtained on free sterols or their trimethylsilyl ethers. Nmr spectra were obtained on a Joelco 100 instrument. Ergosta-7, 22-dien-3β-ol, ergosta-8, 22-dien-3β-ol, ergosta-7, 24(28)-dien-3β-ol, ergosta-8, 24(28)-dien-3β-ol, ergosta-7-en-3β-ol, ergosta-8-en-3β-ol, and 4, 4-dimethylcholesta-8, 24-dien-3β-ol were kind gifts from Dr. A. C. Oehlschlager. Obtusifoliol was a kind gift from Dr. L. J. Goad.

Results. The sterol composition of the wild type, nystatin resistant, and sterol requiring yeast strains is shown in Table I. The major sterol (approximately 60% of the total) in wild type cells was ergosta-7, 22-dien-3β-ol. Mass spectra showed a parent peak at m/e 398, and nmr showed 3 vinylic protons at δ 5.25. Its purity and identity were verified by comparing glc and lc retention times to those of known standards. Lanosterol and 8 other sterols in the parent strain were separated and identified in a similar manner. Ergosterol represented only about 8% of total sterol.

The sterol composition of the nystatin resistant and ergosterol requiring mutant was much simpler (Table I), since lanosterol (Fig. 1, I) and three other sterols essentially accounted for the whole sterol fraction. Lanosterol which was present in much larger amounts than in wild type cells, was identified by glc and lc relative retention times. Compound II, 4, 14-dimethylzymo-

TABLE I

Sterol Composition of Wild Type and Sterol Deficient Mutants of Yeast.

Strain	Sterols[a]
D587-4B (wild type)	Ergosta-7, 22-dien-3β-ol Ergosta-8, 22-dien-3β-ol Episterol Ergosterol Ergosta-7-en-3β-ol Fecosterol Ergosta-7, 22, 24(28)-trien-3β-ol Ergosta-8-en-3β-ol Ergosta-8, 22, 24(28)-trien-3β-ol Unidentified sterol Lanosterol
Nys 1 or Erg2[b]	14-Methyl fecosterol Lanosterol Obtusifoliol 4, 14-Dimethylzymosterol Unidentified sterol

[a] Listed in decreasing order of abundance.

[b] Ergosterol, also present in Erg2 strain, was derived from the growth medium.

sterol (4, 14-dimethylcholesta-8, 24-dien-3β-ol) gave a parent ion at m/e 412. Nmr showed geminal vinyl methyl groups (C-26 and C-27, δ 1.60, 1.68), a vinyl proton (C-24, δ 5.12), and a methyl group at C-4, δ 3.42, doublet, J = 6 Hz (in 0.5 moles of tris (dipivalomethanato) europium per mole of sterol in $CDCl_3$) which collapsed to a singlet, δ 3.42, when the proton at C-4 was irradiated at δ 4.85. Obtusifoliol (III, 4, 14-dimethylergosta-8, 24(28)-dien-3β-ol) had a parent ion at m/e 426. Nmr showed an exomethylene group, δ 4.70, and a methyl group at C-4, δ 3.20, doublet, J = 6 Hz (in 0.5 moles of Eu $(DPM)_3$ per mole of sterol) which collapsed to a singlet, δ 3.20, when the

proton at C-4 was irradiated at δ 4.60. The structure assignment was supported by comparison with an authentic sample in glc and lc retention times. The major sterol of the mutants was 14-methylfecosterol (IV, 14-methylergosta-8, 24(28)-dien-3β-ol). It had a parent ion at m/e 412, an exomethylene group, δ 4.71, and did not have a methyl group at C-4, since the spectrum did not show deshielding by complex formation with europium. Incubation of extracts of the nystatin resistant or ergosterol requiring mutant with [^{14}C]-methyl methionine resulted in formation of labeled obtusifoliol (III) and 14-methylfecosterol (IV).

Discussion. For reasons which are not clear the major sterol of strain D587 is ergosta-7, 22-dien-3β-ol rather than ergosterol, which had previously been found to be the major sterol in wild type yeast. In strain D587 ergosterol comprises less than 10% of total sterols, and the sterol composition (Table I) differs in some respects from that reported in other wild type cells (4, 5).

It is clear from the results discussed above that the methyl group at C-14 of lanosterol (I) was conserved in sterols II - IV which accumulated in the mutants, whereas either one or both of the methyl groups at C-4 were lost. A block in removal of the methyl group at C-14 therefore occurred in the mutants. Blocks in other reactions between lanosterol and ergosterol have recently been reported in nystatin resistant mutants of yeast (6-8).

An alternative route for conversion of lanosterol to ergosterol may occur in yeast by way of intermediates II - IV, whereas in the main pathway the methyl group at C-14 of lanosterol is assumed to be removed first (5, 9). The alternative pathway is supported by in vivo conversion of obtusifoliol (III) to ergosterol (9). The most reasonable sequence for formation of 14-methylfecosterol (IV) is by way I → II →III →IV, assuming that transmethyl-

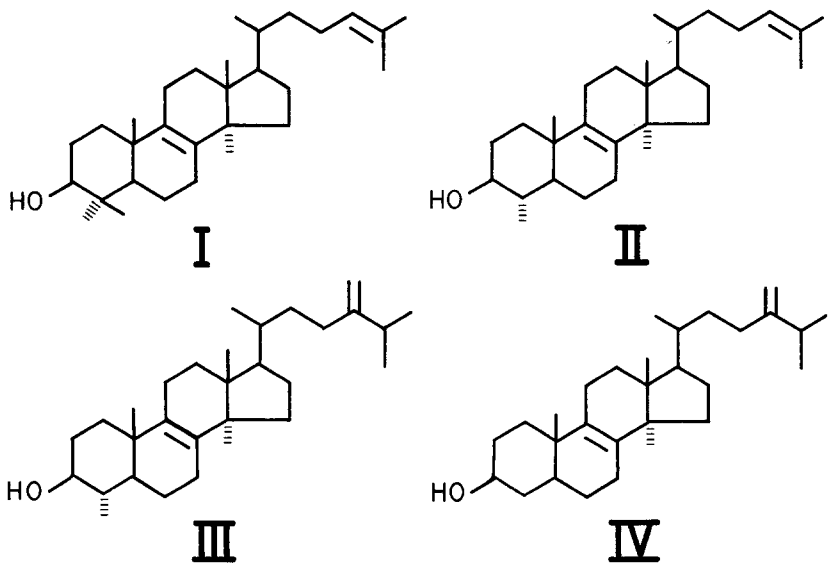

Fig. 1. Sterols composition of nystatin resistant and ergosterol requiring mutants of yeast.

enation of lanosterol did not occur. 4, 14-Dimethylzymosterol, obtusifoliol, and 14-methylfecosterol have not so far been reported in yeast.

The ergosterol requiring mutant had the same sterols, and in approximately the same ratio, but at one-third the level present in the nystatin resistant strain. The lower amount of sterols was probably due to an additional mutation in porphyrin biosynthesis (2), and the likely function of a cytochrome in oxygenation reactions required for removal of sterolic methyl groups (10).

The accumulation of IV suggests that the $\Delta^{8,9}$ isomerase is essentially specific for fecosterol as indicated by previous studies (11). It would also appear that reduction of the $\Delta^{24(28)}$ double bond, and introduction of Δ^{22} double bond in III or IV are restricted in the mutants. Removal of the methyl groups at C-4 and at C-14 of obtusifoliol (III) may therefore constitute the initial reactions in its utilization for ergosterol formation (9), but the order of the reactions cannot be predicted.

Acknowledgments. We are very grateful to Dr. A. C. Oehlschlager for generous gifts of the standard sterols mentioned in the text, and to Dr. L. J. Goad for a generous gift of obtusifoliol. We are pleased to acknowledge the help of Mr. I. Miura with nmr spectra, and the help of Mr. V. Saltomach with mass spectra.

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