Lanosterol Biosynthesis in *Saccharomyces cerevisiae*

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IN a previous publication,[1] it was suggested that the formation of lanosterol from squalene might be accomplished in Nature by a proton-catalysed cyclisation–hydroxylation sequence. Evidence for the latter stage in cell-free yeast system was presented, though a trapping experiment revealed that non-enzyme-bound lanostadiene could not be an intermediate. Recently, the intermediacy of 2,3-oxidosqualene in lanosterol formation in rat liver homogenates has been postulated[2,3] on the basis of compelling experimental evidence. This postulate has been shown to be valid for the biosynthesis of some 3-oxygenated triterpenoids in higher plants[4,5] and in a mould.[6] We now present data to show that the epoxide route is applicable also to yeast sterols.

2,3-Oxidosqualene[7] was degraded to the corresponding C_{27} aldehyde (I) with periodic acid in ether.[8] Squalene was regenerated in 52% overall yield by a Wittig reaction of the aldehyde (I) with [1,3-$^3H_2$]-2-propylidenetriphenylphosphorane, followed by purification *via* the thiourea clathrate. This terminally labelled squalene was converted into 2,3-oxido-[1,24,25,30-$^3H_4$]squalene by the reported procedure.[7]

Lanostadiene was prepared by a modification of the described procedure.[1] Desulphurisation of the ethylenedithioacetal of lanosta-8,24-dien-3-one by W2 or W7 Raney nickel resulted in the formation of lanosta-2,8,24-triene as the major product. A cleaner reduction to lanosta-8,24-diene was effected with lithium in ethylamine at $-20°$ when a 65% yield of hydrocarbon m.p. 83—84° $[\alpha]_D + 65°$ (lit.[9] m.p. 79·5—80°, $[\alpha]_D + 65°$) (both $[\alpha]_D$ in $CHCl_3$) was obtained, after purification by $AgNO_3$–silica gel thick-layer chromatography.

The radioactive precursors were fed to a cell-free system[10] and to whole cells of *Saccharomyces cerevisiae*. The metabolic products were worked-up by dilution with inactive carriers. In an attempt to trap activity at the 2,3-oxidosqualene stage, inactive 2,3-oxidosqualene was fed with the tritiated squalene precursor.

The observed incorporations (see Tables) are consistent with a 2,3-oxidosqualene route to lanosterol (and hence ergosterol) in yeast. There was no observable incorporation of squalene into lanostadiene, but lanostadiene was reproducibly converted into lanosterol, albeit in low yield. It would appear therefore that an oxidative enzyme

TABLE 1

Percentage incorporations in the cell-free system

Precursor	2,3-Oxidosqualene	Lanosta-8,24-diene	Lanosterol
[1,24,25,30-$^3H_4$]Squalene..	—	0·00	0·51[a]
[1,24,25,30-$^3H_4$]Squalene..	1·42[a]	—	0·12[a]
2,3-Oxido[1,24,25,30-$^3H_4$]squalene	—	—	12·3[b]
[2-^{3}H]Lanosta-8,24-diene	—	—	0·06[a]

TABLE 2

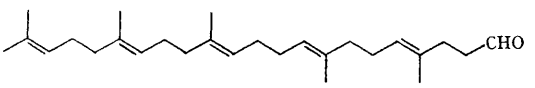
Percentage incorporations in the whole-cell system

Precursor	Lanosterol	Ergosterol
[1,24,25,30-$^3H_4$]Squalene	0·1[a]	1·4[a]
2,3-Oxido[1,24,25,30-$^3H_4$]squalene	11·5[b]	10[b]

[a] Allowing for recovered precursor.
[b] Not allowing for recovered precursor.

in yeast can accept lanostadiene, an apparently unnatural substrate, and effect hydroxylation at C-3. Examples of hydroxylation by micro-organisms are well known,[11] and one other case of C-3 hydroxylation has been reported.[12]

Squalene cyclisation *via* the oxide is thus a general process for polycyclic 3-oxygenated triterpenoid formation. There still remains, however, the possibility that in certain cases, proton-catalysed cyclisation of squalene could occur. Indeed, it is highly probable that 3-deoxygenated triterpenoids are derived by the proton cyclisation route. A proton cyclisation mechanism followed by C-3 hydroxylation is, of course, well established in the chemistry of the diterpenoids.[13]



(I)

Inherent in the synthetic production of labelled squalene and its epoxide are the possibilities for the synthesis of more specifically labelled substrates. The development of these, and their application to the above biosynthetic problems are in progress.

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