Squalene Cyclisation in Yeast

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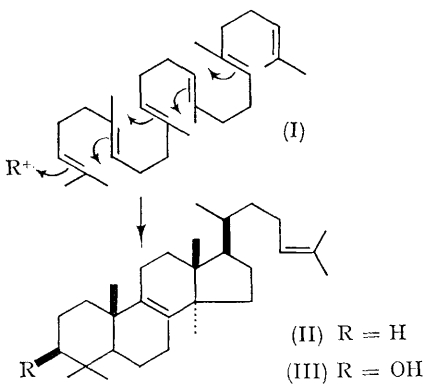
THE biogenetic "isoprene rule" assumes[1] that squalene (I) is cyclised by the biological equivalent of OH^+ to furnish 3-oxygenated triterpenoids like lanosterol (III). Since most diterpenoids and, as many as fifteen[2] triterpenoids, lack oxygen at the 3-position we have for some years felt[3] that all biosynthetic cyclisation of terpene compounds might be initiated by H^+. The oxygen function at C-3 in triterpenoids (and some diterpenoids) would then be introduced at a later stage by hydroxylation.

The evidence is[4] that oxygen is needed for squalene cyclisation and that, in the absence of oxygen, no steroid biosynthesis is detectable. However, if lanosta-8,24-diene (II) were the first formed cyclisation product and if this blocked the enzyme until liberated by the process of C-3 hydroxylation, the experimental facts would still be accommodated by our theory. Other aspects of steroid biosynthesis[5] would remain as before. We have demonstrated that lanosta-8,24-diene *can* be converted into lanosterol by a cell-free yeast system.

Lanosta-8,24-dien-3-one was labelled at the 2-position by base catalysis in tritiated aqueous ethanol. Oxygenation[6] to lanosta-8,24-diene-2,3-dione showed that all the tritium was at C-2 (C-1). The tritiated lanosta-8,24-dien-3-one was converted[7] into its ethylenedithioketal which was desulphurised with Raney nickel to furnish [2-^{3}H]-lanosta-8,24-diene[8] (II).

The latter compound was emulsified in a cell-free system[9] of *Saccharomyces cerevisiae* (Heath and Heather Ltd., St. Albans, Herts) and incubated at

30° for 14 hr. The derived non-saponifiable material was fractionated by thin-layer chromatography. An 0·18% incorporation (allowing for unreacted lanostadiene) into lanosterol (III) was



achieved consistently. The constancy of the radioactivity was demonstrated by acetylation to the acetate and later reconversion into lanosterol. Oxidation to lanosta-8,24-dien-3-one and oxygenation[6] to the 2-keto-derivative confirmed that all the tritium was at C-2 (C-1) and none at C-3. 46% of the lanosta-8,24-diene was recovered unchanged. Although the incorporation into lanosterol is small the results are comparable with those reported earlier in this kind of system for squalene incorporation.[10]

In the presence of inactive lanosta-8,24-diene (4·9 mg.) (±)-[2-^{14}C]mevalonic acid (5μc) was incorporated into lanosterol to the extent of 0·5% (allowing for utilisation of only one optical isomer). The lanosta-8,24-diene recovered showed negligible (<0·006%) incorporation of activity. This shows that, if lanosta-8,24-diene be accepted as an intermediate, it must be attached to the enzyme site in such a way that it cannot be desorbed except by hydroxylation to lanosterol.

Our experiments make it necessary to consider seriously the route squalene → lanostadiene (enzyme bound) → lanosterol for steroid and triterpenoid biosynthesis and we hope to report on more definitive results with other organisms in due course.

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