24-Methylenedihydrolanosterol as a Precursor of Steroids and Triterpenoids

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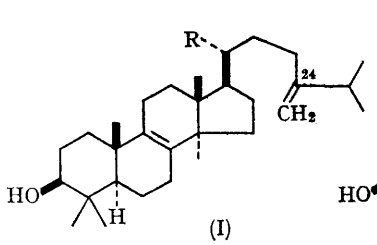
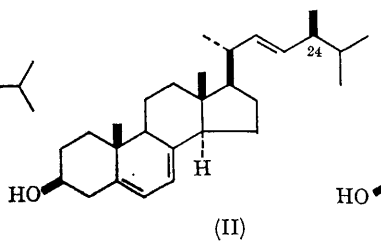
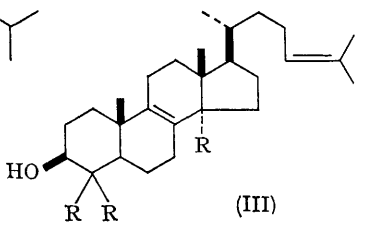
THE biogenetically "extra" carbon atom present in the side-chain of numerous triterpenoids and plant steroids has been shown[1] to be derived from the *S*-methyl of adenosylmethionine[2] with the incorporation of only two of the methyl protons.[3] Several theories have been proposed for the mechanism involved.[4] We report evidence that 24-methylenedihydrolanosterol (I; R = Me) can act as a precursor for ergosterol (II) in yeast and for eburicoic acid (I; R = CO_2H) in *Polyporus sulfureus*.



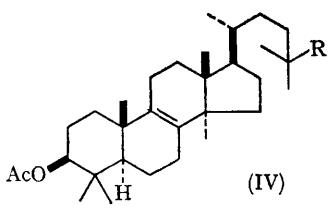
(I)



(II)



(III)



(IV)

Lanosterol acetate monoepoxide,[10] with boron trifluoride in benzene,[11] gave 3β-acetoxy-24-oxolanost-8-ene m.p. 137° $[\alpha]_D^{23}$ 58° and 40% yield of the isomeric aldehyde (IV; R = CHO isolated as the methyl ester of the acid (IV; R = CO_2Me), m.p. 158°, $[\alpha]_D^{26}$ 51·5°. Methyltriphenylphosphonium bromide, labelled by base-catalysed exchange with tritiated water, underwent a Wittig reaction with the above-mentioned ketone to give [28-^{3}H]-24-methylenedihydrolanosterol [eburicol[12] (I; R = Me)].

The common occurrence[5] of triterpenoids like eburicoic acid, and the nonincorporation of zymosterol (III; R = H) into ergosterol (II) by yeast[6] suggests that the "extra" carbon atom referred to above is introduced into lanosterol (III; R = Me) prior to further modification. This view is supported by the isolation of "uncharacterised" steroids in biosynthetic studies[7,8] and by the formation of labelled 24-methylenecycloartanol from labelled methionine in the studies of Ourisson and his colleagues.[1f] Furthermore, the transfer[3b,9] of the C-24 proton of lanosterol to (presumably) C-25 also supports the concept that 24-methylenedihydrolanosterol (I; R = Me) is the first formed alkylated triterpenoid.

This compound was fed to *Saccharomyces cerevisiae* (Heath and Heather Ltd., St. Albans, Herts.) *in vivo* and incubated at 30° for 16 hr. Ergosterol (II) from the nonsaponifiable material showed a 1·2% incorporation (allowing for 26% recovery of precursor). The constancy of the radioactivity was demonstrated by acetylation to the acetate. Ozonolysis gave 2,3-dimethylbutanal from the side chain which retained all of the activity when examined as its dimedone derivative.

[28-^{3}H]-24-Methylenedihydrolanosterol(I; R = Me) was also fed to a surface culture of *Polyporus sulfureus* (Centraalbureau voor Schimmelcultures, Baarn) for five weeks. Eburicoic acid (I; R = CO_2H) was isolated and showed a

0·14% incorporation (allowing for 22% recovery of precursor). Constancy of radioactivity was observed on acetylation to the acetate, methylation to the methyl ester of the acetate, and on hydrolysis to methyl eburicoate.

Our experiments support the alkylation of lanosterol to 24-methylenedihydrolanosterol (possibly *via* 24,25-methylenelanosterol) as a stage in the formation of 24-alkylated triterpenes and steroids. Further work with doubly-labelled 24-methylenedihydrolanosterol is in progress.

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