Terpenoid Biosynthesis: the Stereochemistry of Squalene Cyclisation†

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Summary In the biosynthesis of lanosterol the 4α-methyl group is derived from C-2 of mevalonic acid.

It has been assumed[1] that the enzymatic isomerisation of isopentenyl pyrophosphate to dimethylallyl pyrophosphate proceeds in an analogous manner to the subsequent stages,[2] so that the *trans*-methyl group is derived from C-2 of mevalonic acid. Furthermore it has been assumed[3] that this relationship is maintained during the biosynthesis of the polycyclic terpenoids. The stereospecificity of these

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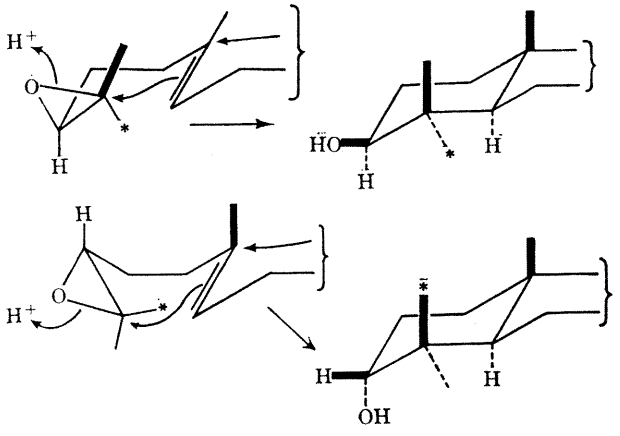
reactions has been clearly demonstrated for several terpenoids[4] including evidence that the triterpenoid substituents at C-4 are stereospecifically labelled[5] by [2-^{14}C]mevalonic acid, and similarly[6] the substituents at C-25. One acyclic case[7] and a limited number of polycyclic terpenoids[8] have been examined and support the above assumptions concerning the stereospecificity of these processes. However, a recent report[9] questions these conclusions.

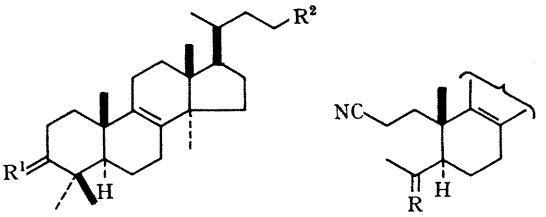
In this report we show that the 4α-methyl group of lanosterol is derived from C-2 of mevalonic acid using a method which is applicable to all triterpenoids with a *gem*-dimethyl group at C-4 and an oxygen function at C-3. Most triterpenoids fulfil these requirements so that the labelling pattern can be used to examine the stereochemistry of the 2,3-oxidosqualene precursor. It is normally assumed (see Scheme) that all triterpenoids are derived from 3*S*-2,3-oxidosqualene giving a 3β-alcohol after cyclisation with the 4α-methyl group derived from C-2 of mevalonic acid. However, a 3*R*-2,3-oxidosqualene would give on cyclisation, via a boat conformation, a 3α-alcohol with the 4β-methyl group derived from C-2 of mevalonic acid (see Scheme).



SCHEME

Several examples of triterpenoids with a 3α-alcohol function are known, especially from the higher plant order *Rutales*.[10]

Three methods were examined for distinguishing between the methyl groups attached to C-4 of the triterpenoid lanosterol (I). Rearrangement of the Baeyer–Villiger oxidation product recently has been shown[11] to be non-stereospecific. Furthermore we were unable to repeat the rearrangement of Kohen and Stevenson;[12] the sole product isolated was the simple elimination product. We have shown[13] that the "abnormal" Beckmann rearrangement is largely stereospecific with the methylene group of the seco-nitrile derived from the 4α-methyl group. This method was applied to lanosterol.



(I) R^1=α—H, β—OH; R^2=CH:CMe_2 (III) R=CH_2
(II) R^1=NOH; R^2=CH_2CHMe_2 (IV) R=O

Labelled lanosterol (I) was prepared from [2-^{14}C]mevalonic acid with an arsenate-inhibited rat liver homogenate.[14] The lanosterol was diluted with inactive material, hydrogenated, oxidised, and treated with hydroxylamine to give the 3-oxime (II) m.p. 172—173° (1·82 c.p.s./μmole). Rearrangement of the oxime, and cleavage of the generated double bond of the oily seco-nitrile III with osmium tetroxide and lead tetra-acetate gave the keto nitrile IV m.p. 77—80° (1·62 c.p.s./μmole, calc. 1·52 c.p.s./μmole), and formaldehyde isolated as its dimedone derivative (0·21 c.p.s./μmole, calc. 0·30 c.p.s./μmole). The calculated figures quoted above are based on a completely stereospecific reaction. In fact this is not completely true. Clearly the 4α-methyl group of lanosterol is derived from C-2 of mevalonic acid, but the "abnormal" Beckmann rearrangement is only 70% stereospecific. A similar result was obtained from the model experiments.[13]

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