Terpenoid Biosynthesis: the Stereochemistry of Squalene Cyclisation†

By G. P. Moss* and S. A. NICOLAIDIS

(*Department of Chemistry, Queen Mary College, Mile End Road, London, E.*1)

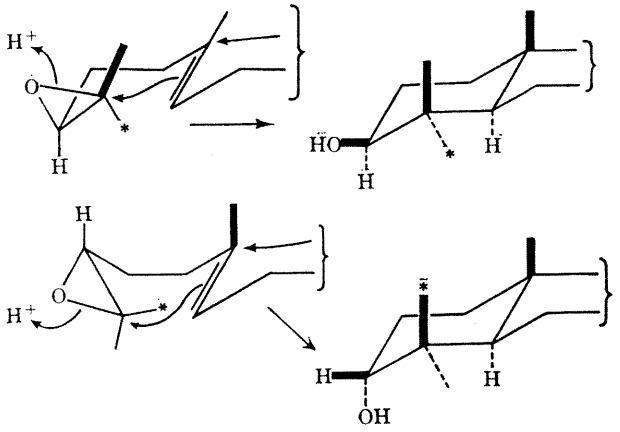
Summary In the biosynthesis of lanosterol the 4α-methyl group is derived from C-2 of mevalonic acid.

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

† Part of this work was presented at the 5th International IUPAC Symposium on the Chemistry of Natural Products held in London, July 1968.

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.

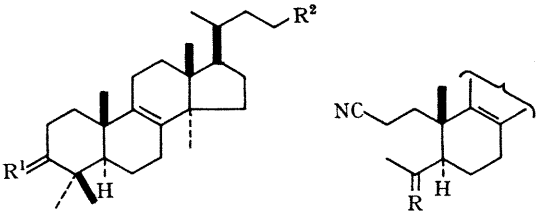
In this report we show that the 4α-methyl group of lanosterol is derived from C-2 of mevalonic acid using a method which is applicable to all triterpenoids with a *gem*-dimethyl group at C-4 and an oxygen function at C-3. Most triterpenoids fulfil these requirements so that the labelling pattern can 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]

(*Received, July 25th*, 1969; *Com.* 1133.)

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