Stereochemistry of a Migrating Methyl Group during the Biosynthesis of Lanosterol

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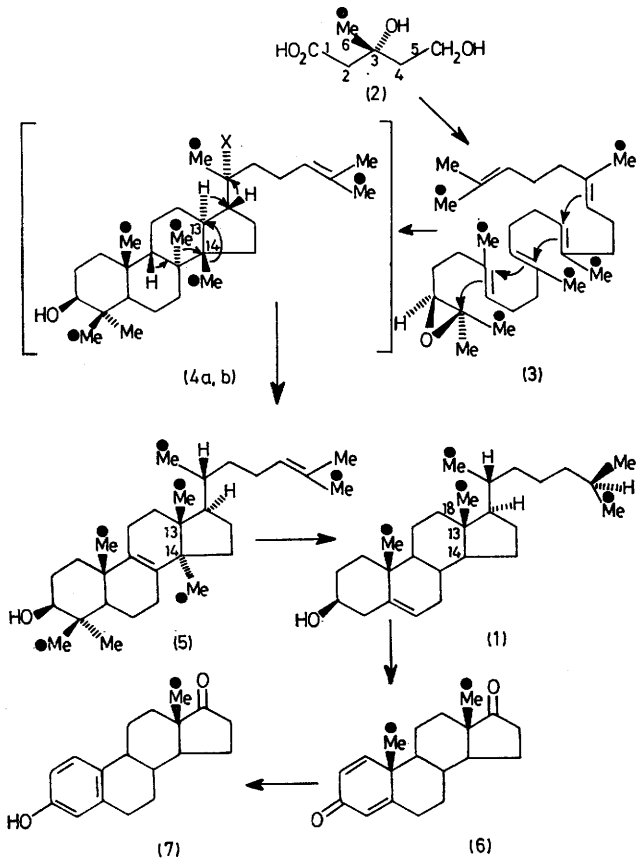
Summary The methyl group becomes attached to C-13 of cholesterol by intramolecular migration without configurational change.

THE methyl group at C-13 of cholesterol (**1**) arrives there by rearrangement of a precursor. This rearrangement is intramolecular and occurs within a structure derived from a single molecule of mevalonic acid (**2**).[1] The preferred mechanism for biosynthesis of the sterol skeleton at present postulates cyclisation of squalene epoxide (**3**) to a tetracyclic entity (**4a**)[2] or (**4b**),[3] followed by a sequence of 1,2-shifts terminated by expulsion of the C-9β proton to form lanosterol (**5**). The methyl migration in question is then between C-14 and C-13 of the precursor (**4a** or **4b**) and it might *prima facie* occur either with retention or with inversion of configuration of the methyl group. If migration is with inversion, the chirality of a chiral methyl group at C-6 of mevalonate (**2**) will be inverted after conversion

into a C-18 methyl group of cholesterol; if migration is with retention, the chirality will be unchanged.



(4a) X = positive charge
(4b) X = nucleophilic group on 2,3-oxidosqualene cyclase.
● Me = chiral methyl

SCHEME

Mevalonolactones, chiral at C-6, were synthesised by separately converting *R*- and *S*-[$^2H_1$,$^3H_1$]potassium acetate into the tetraethylammonium salt which was condensed with benzyl chloride to give benzyl acetate (*cf.* ref. 4). 4-Methylhepta-1,6-dien-4-ol was synthesised from allylmagnesium bromide and benzyl acetate and was converted into 3-hydroxy-3-methylglutaric acid by oxidative ozonolysis.[5] The acid was reduced with borane[6] in tetrahydrofuran to 3-methyl-pentane-1,3,5-triol which was oxidised with silver carbonate–Celite[7] to give mevalonolactone. The chiral mevalonates, purified by chromatography on silicic acid, gave satisfactory analysis on g.l.c., t.l.c., and m.s., and a constant $^3H/^{14}C$ ratio for the benzhydrylamides.

The (3*RS*)-[6-^{14}C,(6*R*)-6-$^2H_1$,$^3H_1$]- and (3*RS*)-[6-^{14}C,(6*S*)-6-$^2H_1$,$^3H_1$]-mevalonates (**2**) were each converted into cholesterol (**1**) by a rat liver preparation.[8] The cholesterol (**1**) was degraded to androsta-1,4-diene-3,17-dione (**6**) by microbiological transformation[8] using *Mycobacterium phlei*. Androsta-1,4-diene-3-one-17-ethylene acetal was converted into oestrone (**7**) by reductive aromatisation with lithium, biphenyl, and diphenylmethane in tetrahydrofuran.[9] The oestrone (**7**) was purified by chromatography on Sephadex LH20 and by sublimation before being oxidised with CrO_3[1] to acetic acid which was recovered by steam distillation and purified by partition chromatography.

The chirality of the acetic acid was determined as previously described;[10] acetate was converted into (2*S*)-malate, which was equilibrated with fumarate hydratase and the loss of tritium measured. Acetates of *R*- and *S*-chirality gave malates which respectively retained 76 and 24% of their tritium after treatment with fumarate hydratase.

The conversion of (3*RS*)-[6-^{14}C,(6*R*)-6-$^2H_1$,$^3H_1$]mevalonate into cholesterol (**1**) and oestrone (**7**) gave acetate which yielded a (2*S*)-malate that retained 68·6% of its tritium after treatment with fumarate hydratase; thus the configuration of this acetate was *R*. (3*RS*)-[6-^{14}C,(6*S*)-6-$^2H_1$,$^3H_1$] mevalonate when transformed in the same way gave malate that retained 31·9% of its tritium; thus the configuration of this acetate was *S*. There has been no overall change in configuration in this methyl group between C-6 of mevalonate (**2**) and C-18 of cholesterol (**1**) and migration from 'C-14' to 'C-13' of a lanosterol precursor therefore occurs with retention of configuration, as seems to be the rule with non-enzymic carbonium-ion rearrangements.[11]

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