Stereochemistry of Sterol Biosynthesis: Interrelationship of the Terminal Methyl Groups in Squalene, the C-1,1′ Methyls in Squalene 2,3-Oxide, and the C-30,31 Methyls in Lanosterol

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Summary The *trans*-terminal methyls of squalene derive from C-2 of mevalonic acid, and the conversion of squalene in turn into squalene 2,3-oxide and lanosterol occurs without interchange of isopropylidene methyls, so that C-2 of mevalonic acid provides the 4α-methyl group of lanosterol.

IT has been assumed[1] that the gem-dimethyl substituents (C-30, C-31) at C-4 in lanosterol derive from the terminal methyls of squalene in a fashion stereochemically analogous to that demonstrated for the corresponding carbons (4α-methyl and 4β-hydroxymethyl) of soyasapogenol. However, recently reported findings[2] in our laboratory have brought this assumption into question, and a definitive study of these relationships in lanosterol biosynthesis was lacking. Moreover, the stereochemistry of these processes bears upon the mechanism of biosynthesis of squalene 2,3-oxide from squalene, and of its cyclization to lanosterol.[3] In this report we show unambiguously that (1) in the terminal isopropylidene groups of squalene, C-2 of mevalonic acid provides (*via* C-4 of isopentenyl pyrophosphate) that methyl group which lies *trans* to the remainder of the squalene chain; (2) the biological conversion of squalene into squalene 2,3-oxide, and (3) the cyclization of the 2,3-oxide to lanosterol take place in turn with no change in these steric relationships, so that the 4α-methyl group of lanosterol is, in fact, derived from C-2 of mevalonic acid.

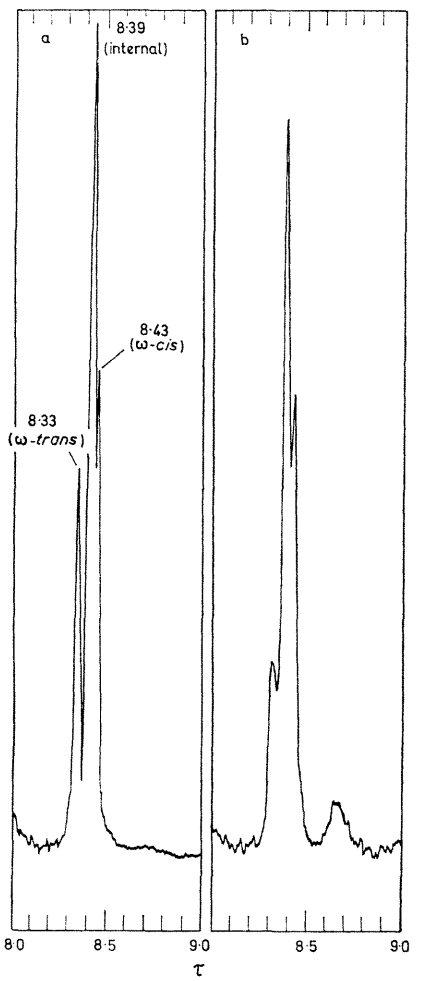

FIGURE 1. *Segments of n.m.r. spectra of* (a) *normal,* (b) *biosynthetic deuteriated squalenes.*

We have made use of the fact that isopentenyl pyrophosphate is enzymically isomerized to dimethylallyl pyrophosphate with the loss of a proton from C-2 and incorporation at C-4 of a proton from the aqueous medium, so that squalene biosynthesized in a medium enriched in D_2O becomes labelled with deuterium in the terminal methyl groups[4] derived from the C-4 methylene of isopentenyl pyrophosphate (C-2 of mevalonic acid). The resultant distribution of deuterium in squalene and lanosterol has been analysed by n.m.r. spectroscopy.

[^{14}C–D]-Squalene was biosynthesized by anaerobic incubation of [2-^{14}C] mevalonic acid with the microsomes and supernatant fraction obtained by centrifugation (20 min. at 13,000 × *g*) of a homogenate of rat liver prepared in 99·8% D_2O, followed by purification by t.l.c.[3a] The product gave the expected mass-spectral pattern with a molecular ion at *m/e* 428 and yielded an n.m.r. spectrum, the relevant portion of which is shown in Figure 1b. The corresponding portion of the n.m.r. spectrum for normal squalene is shown in Figure 1a. A portion of the deuteriated product was converted into squalene 2,3-oxide by aerobic incubation with a rat liver microsome and supernatant preparation in the presence of 2,3-imino-squalene (an inhibitor of cyclization)[5] followed by saponification, extraction, and purification by t.l.c.[3a] A second portion was converted chemically into squalene 2,3-oxide *via* the bromohydrin[6] and also purified by t.l.c.[3a] Both the biochemically and chemically formed 2,3-oxides were examined by n.m.r. (Figures 2b and 2c, respectively), and for comparison the n.m.r. spectrum of unlabelled, chemically prepared squalene 2,3-oxide was measured (Figure 2a). The biologically derived deuteriated oxide was further converted into lanosterol by the action of a clarified squalene 2,3-oxide, lanosterol cyclase preparation obtained from rat liver microsomes as indicated elsewhere.[7] This lanosterol, isolated in the usual way and purified by t.l.c.,[3a] was examined by n.m.r. (Figure 3b) and the n.m.r. spectrum of normal lanosterol (Figure 3a) was also recorded. All n.m.r. spectra were recorded by means of a 100 MHz

Varian HA 100 instrument with perdeuteriobenzene as solvent.

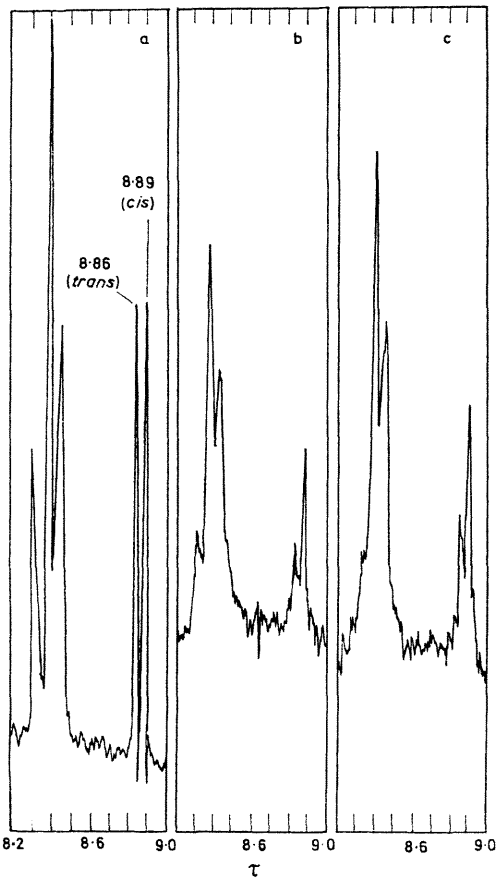

FIGURE 2. *Segments of n.m.r. spectra of* (a) *normal,* (b) *biosynthetic (deuteriated) and* (c) *chemically prepared (deuteriated) squalene* 2,3-*oxides.*

Figures 1a and 1b show three peaks in the n.m.r. spectra of normal and biosynthetically deuteriated squalenes at τ 8·33 and 8·43 corresponding to the resonances of protons of the terminal methyls *trans* and *cis* respectively to the carbon chain, and a strong peak at τ 8·39 corresponding to the internal methyls, lying *cis* to the chain.[8] In comparison with the normal sample (1a), the peak at τ 8·33 in the case of the deuteriated sample (Ib) is depressed to less than half its intensity relative to the peak at τ 8·43. This is the expected result if, as suggested by Cornforth *et al.*,[9] the stereochemistry of isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate is analogous to that of the condensation (with concomitant loss of a proton) of an allyl pyrophosphate with isopentenyl pyrophosphate. The n.m.r. spectra of the three samples of squalene 2,3-oxide (Figures 2a, b, c) also showed the peaks at τ 8·33, 8.43, and 8·39, corresponding to isopropylidene and internal methyls, and in addition showed strong peaks at τ 8·84 and 8·89 corresponding to the protons of methyls, respectively *trans* and *cis* to the chain. In support of these assignments the peak at τ 8·89 is lower than that at 8·84, due to coupling of the methyl *cis* to the chain with the hydrogen at C-3. It is evident that the peak at τ 8·84 is markedly reduced in intensity in both the biochemically and synthetically prepared deuteriated oxides (Figures 2b and 2c, respectively). It follows that the orientation of the deuteriated terminal methyl is the same in both; and since the chemical method of preparing the oxide *via* the bromohydrin entails no net inversion of the methyl groups, this must also be true of the biological oxidation.

Figures 3a and 3b, respectively, illustrate expanded segments of the n.m.r. spectra of unlabelled lanosterol and

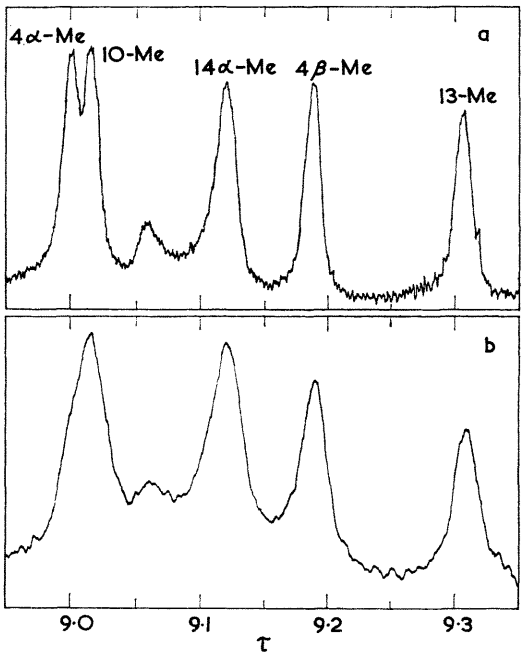

FIGURE 3. *Expanded segments of the n.m.r. spectra of* (a) *normal lanosterol and* (b) *lanosterol obtained by enzymic cyclization of biosynthetic deuteriated squalene* 2,3-*oxide.*

of lanosterol obtained by enzymic cyclization of the biologically prepared deuteriated squalene 2,3-oxide. The indicated assignments are based upon published precedents.[10] The resonance at τ 9·00 (4α-methyl) is strongly suppressed in the deuteriated sample, which demonstrates that squalene oxide cyclizes to lanosterol without interchange of the terminal methyl substituents.

The stereochemical derivation of the terminal methyl groups of squalene from mevalonic acid in the mammalian enzyme system, as revealed by this study, corresponds with that assumed on the basis of studies with soyasapogenol, a cyclic terpenoid of vegetable origin. The oxide is formed with no inversion of the terminal *gem*-dimethyl group. Thus its mechanism of formation is probably analogous to that of various steroid oxides,[11] in which inversion cannot occur, and of *trans*-succinic oxide from fumarate in *Aspergillus fumigatus*.[12] The cyclization of the oxide without interchange of terminal methyls accords with the view that the enzymic attack on the oxide ring of squalene 2,3-oxide and the formation of ring A of lanosterol are parts of a concerted process for which non-enzymic analogies have previously been discussed.[3a,13] However, it must be recognized that the results do not rule

out an even number of inversions in any of the steps discussed, or the possibility that the first-formed carbonium ion is prevented from rotation by a specific interaction with the enzyme active site.

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