# The Stereochemistry of Hydrogen Elimination at C-7 during Cholesterol Biosynthesis in Rat Liver

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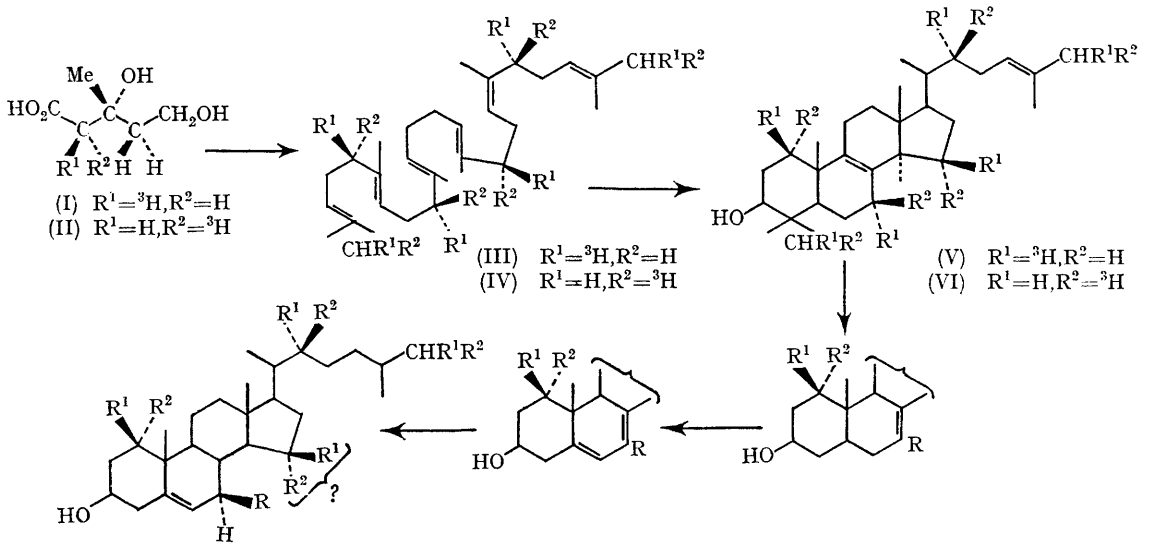
THE biosynthesis of cholesterol from lanosterol involves, among other changes, migration of the $\Delta^8$ bond to $\Delta^7$, formation of 7-dehydrocholesterol, and subsequent reduction of the $\Delta^7$ bond to give cholesterol.[1] The enzyme-catalysed conversion of $\Delta^8$-sterols into $\Delta^7$-sterols has been reported[2] and loss of a hydrogen atom from C-7 has been observed during conversion of lanosterol into cholesterol.[3] We now describe the stereochemistry of the hydrogen elimination from C-7 during cholesterol formation by a rat liver system.

Cornforth, Popják, and their co-workers[4] have shown that incubation of (3*R*)-[2-$^{14}$C-(2*R*)-2-$^3H_1$]-mevalonic acid (MVA), (I), or (3*R*)-[2-$^{14}$C-(2*S*)-2-$^3H_1$]-MVA, (II), with a rat liver preparation will produce squalene (III) or (IV), respectively, in which the stereospecific tritium labelling is retained. Cyclisation of (III) or (IV) by the generally accepted mechanism[5] will give lanosterol (V) or (VI) respectively, again stereospecifically labelled with tritium. In particular, the lanosterol will have tritium label in either the 7$\alpha$ (V) or the 7$\beta$ (VI) position. Enzymic conversion of (I) or (II) into cholesterol followed by location of tritium at C-7 therefore provided the method used in the present work for the examination of C-7 hydrogen elimination during $\Delta^7$ bond formation.

(3*R*)-[2-$^{14}$C-(2*R*)-2-$^3H_1$]-MVA, (I), (1·0 $\mu$c of $^{14}$C; $^3$H:$^{14}$C = 9·40) was incubated for 3 hr. at 37° with a rat liver homogenate[6] and the non-saponifiable lipid isolated (8·83 × $10^5$ d.p.m. of $^{14}$C). Thin-layer chromatography (t.l.c.) on silica gel gave squalene (2·32 × $10^3$ d.p.m. of $^{14}$C) and cholesterol (4·91 × $10^5$ d.p.m. of $^{14}$C). The squalene ($^3$H : $^{14}$C = 8·75)† was further purified by a second t.l.c. separation whilst the cholesterol fraction was acetylated and

† The small decrease in the $^3$H : $^{14}$C ratio upon conversion of the doubly labelled MVA into squalene can be explained by loss of tritium caused by the reversibility of the isopentenyl pyrophosphate–dimethyl allyl pyrophosphate isomerase reaction (P. W. Holloway and G. Popják, *Biochem. J.*, 1968, **106**, 835); the effect was more marked in *Ochromonas malhamensis* (reference 10).



(I) $R^1={}^3H, R^2=H$
(II) $R^1=H, R^2={}^3H$

(III) $R^1={}^3H, R^2=H$
(IV) $R^1=H, R^2={}^3H$

(V) $R^1={}^3H, R^2=H$
(VI) $R^1=H, R^2={}^3H$

(VII) $R={}^3H, R^1={}^3H, R^2=H$
(VIII) $R=H, R^1=H, R^2={}^3H$

purified by t.l.c. on $AgNO_3$–silica gel to give cholesteryl acetate ($3{\cdot}83 \times 10^5$ d.p.m. of $^{14}C$, $^3H:^{14}C = 8{\cdot}71$). Addition of carrier cholesteryl acetate (1·99 g.) followed by two crystallisations gave 163 d.p.m. of $^{14}C$/mg., $^3H:^{14}C = 8{\cdot}69$. Oxidation[7] of the cholesteryl acetate produced 3β-acetoxycholest-5-en-7-one, m.p. 158—159°, which was purified by chromatography on alumina followed by two crystallisations; 162 d.p.m. of $^{14}C$/mg., $^3H:^{14}C = 7{\cdot}06$. With the observed $^3H:^{14}C$ ratio of squalene (8·75) corresponding to a $^3H:^{14}C$ atomic ratio of 6 : 6, the corrected $^3H:^{14}C$ atomic ratio for the cholesteryl acetate is 4·97 : 5, whilst the corrected $^3H:^{14}C$ atomic ratio for the 3β-acetoxycholest-5-en-7-one is 4·03 : 5. This decrease in the $^3H:^{14}C$ ratio therefore demonstrates the presence of a tritium atom at C-7 of the cholesterol and hence the retention of the 2-*pro-R* hydrogen of mevalonic acid during its incorporation into cholesterol. This corresponds to the retention of the 7α-tritium of lanosterol (V) during its enzymic conversion into cholesterol (VII).

A portion (110·8 mg.) of the doubly labelled cholesterol from the above incubation was converted into cholest-6-en-5α-hydroperoxide-3β-ol,[8] m.p. 146—147°, 163 d.p.m./mg., $^3H:^{14}C = 8{\cdot}83$; corrected $^3H:^{14}C = 5{\cdot}05 : 5$. This demonstrates that the tritium atom retained at C-7 had the 7β-configuration[8] and thus confirms previous evidence[9] that reduction of the $\Delta^7$ bond involves hydrogen addition at C-7 from the α face of ring B.

In a similar experiment (3*R*)-[2-$^{14}$C-(2*S*)-2-$^3H_1$]-MVA, (II) (1·0 μc of $^{14}C$, $^3H:^{14}C = 9{\cdot}30$) was incubated with a rat liver homogenate and gave the following results: non-saponifiable lipid, $5{\cdot}95 \times 10^5$ d.p.m. of $^{14}C$; squalene, $7{\cdot}58 \times 10^2$ d.p.m. of $^{14}C$, $^3H:^{14}C = 8{\cdot}45$†; cholesterol fraction, $3{\cdot}44 \times 10^5$ d.p.m. of $^{14}C$. Cholesteryl acetate purified by $AgNO_3$–silica gel t.l.c. contained $2{\cdot}53 \times 10^5$ d.p.m. of $^{14}C$, $^3H:^{14}C = 5{\cdot}65$. Two crystallisations after addition of carrier cholesteryl acetate (2·01 g.) gave 106 d.p.m. of $^{14}C$/mg., $^3H:^{14}C = 5{\cdot}53$, and oxidation produced 3β-acetoxycholest-5-en-7-one, 105 d.p.m. of $^{14}C$/mg., $^3H:^{14}C = 5{\cdot}37$. The virtually unchanged $^3H:^{14}C$ ratio upon formation of the 7-ketone demonstrates the absence of tritium at C-7 of the biosynthesised cholesterol and shows that the 2-*pro-S* hydrogen of MVA incorporated at C-7 of a cholesterol precursor is eliminated, *i.e.* the 7β-tritium atom is lost during the enzymatic conversion of lanosterol, (VI), into cholesterol, (VIII). The results of the two experiments just described are therefore complementary and establish that during cholesterol biosynthesis by a rat liver system the 7β-hydrogen atom of a precursor sterol is eliminated. This is in agreement with our previous results obtained for poriferasterol biosynthesis in *Ochromonas malhamensis*.[10] The C-7 hydrogen elimination most probably occurs as a result of migration of the $\Delta^8$ bond to the $\Delta^7$ position in the appropriate sterol intermediates.

Consideration of the results obtained from the

(3*R*)-[2-$^{14}$C-(2*S*)-2-$^{3}H_1$]-MVA, (II), incubation reveals that conversion of the observed $^{3}H:^{14}C$ ratio of the cholesterol to the corrected $^{3}H:^{14}C$ atomic ratio (based upon squalene) gives a value of 3·27 : 5 instead of the anticipated ratio of 4 : 5. This result, which has been confirmed in three separate experiments, indicates that in addition to the C-7 hydrogen, a second hydrogen atom derived from the 2-*pro*-*S* position of MVA is lost during conversion of squalene into cholesterol. Preliminary work suggests that the second tritium atom is lost from C-15 and work is now in progress to establish this point.

We thank the S.R.C. for financial support, and for a Research Studentship (to G.F.G.).

(*Received, July* 15*th*, 1968; *Com.* 945.)

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