The Incorporation of a 15β-Hydrogen Atom from the Medium in Cholesterol Biosynthesis

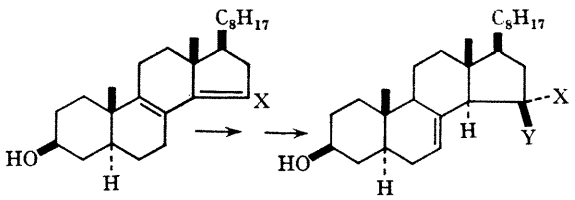
By M. AKHTAR,* A. D. RAHIMTULA, and D. C. WILTON

(*Department of Physiology and Biochemistry, The University of Southampton, Southampton* SO9 5NH)

Summary The reduction of the 14(15)-double bond in the biological conversion of cholesta-8,14-dien-3β-ol into cholest-7-en-3β-ol occurs *via* a *trans*-addition, the 14α-hydrogen being derived from the 4-position of NADPH, the 15β-hydrogen from a proton source.

IT has recently been shown that the biosynthesis of cholesterol from lanosterol involves the loss of the 15α-hydrogen atom[1–3] and occurs through the intermediacy of an 8,14-diene system.[4] In the further conversion of the 8,14-diene (III) into cholesterol a hydrogen atom from the 4-position of NADPH is incoprorated at C-14α and another from a proton source at C-15.[5] We now show that the proton-source derived hydrogen atom at C-15 has the β-configuration. The stereochemical assignment has been made possible by the discovery that the pyrolytic conversion of the 3β,7α-dibenzoate (VI) into the 7,14-diene (VII) occurs predominantly with the loss of a 15α-hydrogen atom.

The stereochemical studies were carried out on three samples of cholest-7-en-3β-ol (I). The first sample was prepared by the incubation of non-radioactive cholesta-8,14-dien-3β-ol (III) with a 10,000 *g* supernatant of rat liver homogenate[6] in the presence of tritiated water. The cholest-7-ene-3β-ol (I) thus prepared should contain radioactivity



(III) (I)

Sample 1 (I; X = H, Y = T) *prepared enzymically from* (III; X = H) *and* T_2O.

Sample 2 (I; X = T, Y = H) *prepared enzymically from* (III; X = T) *and* H_2O.

Sample 3 (I; X = T, Y = H) *prepared by the catalytic reduction of* (III; X = H) *with tritium gas.*

at C-14α† and C-15 due to the reduction of the 14(15)-double bond (reaction i) and at C-9 due to the isomerization reaction (reaction ii)[5] (Scheme).

$$\text{8,14-diene} \xrightarrow[\text{anaerobic NADPH}]{\text{(i)}} \text{8-ene} \xrightarrow[\text{anaerobic}]{\text{(ii)}} \textbf{7-ene}$$

SCHEME

† Due to the NADPH—H_2O equilibrating enzyme system.[6,12]

The second sample was prepared as above except that in this case, the substrate [15-$^3H_1$]-cholesta-8,14-dien-3β-ol (III)‡ contained radioactivity at C-15 and the medium contained no isotopic hydrogen. The third sample of cholest-7-en-3β-ol (I) was prepared by the catalytic reduction of non-radioactive cholesta-8,14-dien-3β-ol (III) with tritium gas (generated from tritiated water and lithium metal). The resulting cholest-8-en-3β-ol§ was isomerized enzymically as above to a mixture of cholest-8-en-3β-ol and cholest-7-en-3β-ol (I).

TABLE

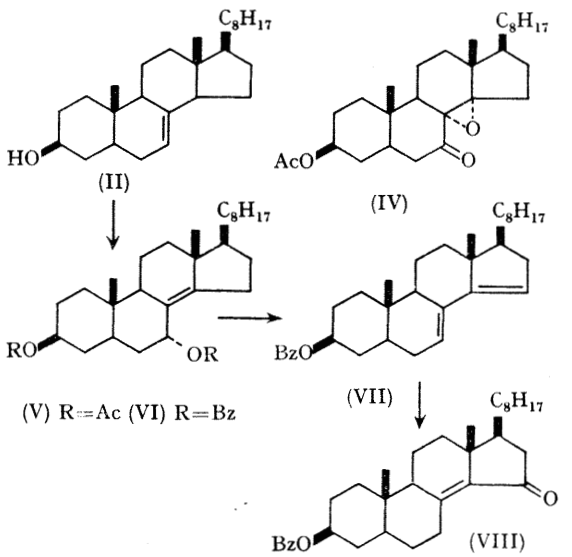
Degradation of the three samples of cholest-7-en-3β-ol (I).

	Radioactivity in		
	Sample 1	Sample 2 (c.p.m./mmole)	Sample 3
Cholest-7-en-3β-ol (I)	160,470	49,700	38,070
8,14-Epoxide (IV)	141,370	49,650	26,000
7,14-Diene (VII)	139,880	37,800	18,000
15-Ketone (VIII)	74,470	29,526	11,150
Total amount of tritium at C-15	66,630	20,174	14,850
Tritium lost from C-15 during the pyrolysis ..	1490	11,900	8000
% of tritium lost from C-15 during the pyrolysis	3%	59%	54%

The three samples of cholest-7-en-3β-ol (I), which contained radioactivity at C-15 and varying amounts of cholest-8-en-3β-ol as a contaminant, were subjected to two series of degradations. Firstly, cholest-7-en-3β-ol (I) was oxidized with CrO_3–HOAc[7] to the 8,14-epoxide (IV). The latter compound arises only from cholest-7-en-3β-ol (I); the impurity of cholest-8-en-3β-ol does not interfere with this conversion. Secondly, cholest-7-en-3β-ol (I) was oxidized with SeO_2–HOAc[8] to the 3β,7α-diacetate (V). The latter compound (V) was converted into the 3β,7α-dibenzoate (VI) which on pyrolysis in dimethylaniline[8] furnished the 7,14-diene (VII). The latter compound (VII), once again, arises only from cholest-7-en-3β-ol (I) and not from cholest-8-en-3β-ol. The diene (VII) was converted into the 15-ketone (VIII).[9]

The total amount of radioactivity at C-15 of cholest-7-en-3β-ol (I) = specific activity of the 8,14-epoxide (IV) minus the specific activity of the 15-ketone (VIII).

Similarly, the amount of radioactivity removed from C-15 in the pyrolytic conversion of the dibenzoate (VI) into the 7,14-diene (VII) = specific activity of the 8,14-epoxide (IV) minus the specific activity of the 7,14-diene (VII). The results in the Table show that sample 1, which was biosynthesized from non-radioactive cholesta-8,14-dien-3β-ol (III) in the presence of tritiated water, lost less than 3% of the total radioactivity present at C-15 in the conversion of the 3β,7α-dibenzoate (VI) into the 7,14-diene (VII). However, sample 2, which was biosynthesized from [15-$^3H_1$]-cholesta-8,14-dien-3β-ol (III) in a non-isotopic medium, lost 59% of the total radioactivity present at C-15 in the same conversion. These experiments show that the two biosynthetic samples contain radioactivity at C-15 associated with opposite orientations and that the pyrolytic conversion of the 3β,7α-dibenzoate (VI) into the 7,14-diene (VII) occurs with a great deal of stereospecificity. Sample 3, which was obtained by the catalytic reduction of non-radioactive cholesta-8,14-dien-3β-ol (III) with tritium gas and which should contain the labelled hydrogen atom in the 15α-orientation, lost 54% of the radioactivity in the pyrolytic step. Assuming that the catalytic hydrogenation results in the *cis*-addition of hydrogen atoms at C-14α and C-15α, the similarity in the loss of tritium during the pyrolysis in sample 2 and 3 suggests that the tritium atom in the biosynthetic sample 2 has the 15α-orientation. These results



‡ [15-$^3H_1$]-Cholesta-8,14-dien-3β-ol (III) was prepared by the acid equilibration of non-radioactive cholesta-8,14-dien-3β-ol(III) (as the acetate) under the same conditions used by Gaustchi and Bloch[11] for the conversion of 4,4-dimethylcholesta-5,7-dien-3β-ol acetate into 4,4-dimethylcholesta-8,14-dien-3β-ol acetate except that a trace of tritiated water was included. [15-$^3H_1$]-Cholesta-8,14-dien-3β-ol (III) so prepared contains about 40% of the total radioactivity at C-15. The remaining radioactivity was not present at C-7 or C-16. The absence of radioactivity at C-16 is deduced from the knowledge that, under identical conditions, cholesta-5,7-dien-3β-ol acetate containing tritium at C-16 (biosynthesized from [5*RS*-$^3H_2$]-MVA) lost no radioactivity from C-16 when rearranged into cholesta-8,14-dien-3β-ol acetate.

§ [14α,15α-$^3H_2$]-Cholest-8-en-4β-ol contains up to 71% of the total radioactivity at C-14α and C-15α. A part of the remaining radioactivity could be present at C-16. The total radioactivity at C-15 is obtained from the specific activity of the 15-ketone (VIII). It is quite possible that in the preparation of this compound (VIII) some of the radioactivity, if present at C-16, will also be removed. This, however, will not affect the main conclusions of this paper. In such an event the 54% of the radioactivity removed in the conversion of (VI) into (VIII) will be an underestimation.

are valid only as far as they give information on the percentage of the total radioactivity at C-15 removed in the pyrolysis step. The degradation of the three samples of cholest-7-en-3β-ol (I) was repeated twice with essentially similar results. The fact that when tritium is located at C-15β the conversion of the dibenzoate (VI) into the 7,14-diene (VII) occurs with the exclusive loss of the 15α-hydrogen atom whereas the presence of tritium at C-15α results in only a 60% loss of label may be attributed to the alteration of the stereospecificity of elimination by the presence of hydrogen isotopes.¶

We therefore infer that the reduction of the 14(15)-double bond in the biological conversion of cholesta-8,14-dien-3β-ol (III) into cholest-7-en-3β-ol (I) occurs through a *trans*-addition, the 14α-hydrogen atom being derived from the 4-position of NADPH[5] and the 15β-hydrogen atom from a proton source. Similar observations on the reduction of the 7(8)-double bond in the conversion of cholesta-5,7-dien-3β-ol into cholesterol have been published.[6] These results, together with the previous observations from this[3] and other[1,2] laboratories also suggest that in the biosynthesis of cholesterol from lanosterol, the 15α-hydrogen atom of the latter is lost and that its 15β-hydrogen atom becomes the 15α-hydrogen atom of cholesterol. Recently Caspi *et al.*,[10] using a different approach, have arrived at the same conclusion.

(*Received, August 11th*, 1969; *Com.* 1228.)

¶ We thank the referee for prompting us to insert this view and for pointing out that related behaviour has been observed by Cornforth *et al.*[13]

[1] L. Canonica, A. Fiecchi, M. G. Kienle, A. Scala, G. Galli, E. G. Paoletti, and R. Paoletti, *J. Amer. Chem. Soc.*, 1968, **90**, 3597.
[2] G. F. Gibbons, L. J. Goad, and T. W. Goodwin, *Chem. Comm.*, 1968, 1458.
[3] M. Akhtar, A. D. Rahimtula, I. A. Watkinson, D. C. Wilton, and K. A. Munday, *European J. Biochem.*, 1969, **9**, 107.
[4] I. A. Watkinson and M. Akhtar, *Chem. Comm.*, 1969, 206.
[5] M. Akhtar, A. D. Rahimtula, I. A. Watkinson, D. C. Wilton, and K. A. Munday, *Chem. Comm.*, 1969, 149.
[6] D. C. Wilton, K. A. Munday, S. J. M. Skinner, and M. Akhtar, *Biochem. J.*, 1968, **106**, 803.
[7] L. F. Fieser, *J. Amer. Chem. Soc.*, 1953, **75**, 4395.
[8] L. F. Fieser and G. Ourisson, *J. Amer. Chem. Soc.*, 1953, **75**, 4404.
[9] J. C. Knight, P. D. Klein, and P. Z. Szezepanik, *J. Biol. Chem.*, 1966, **241**, 1502.
[10] E. Caspi, P. J. Ramm, and R. E. Gain, *J. Amer. Chem. Soc.*, 1969, **91**, 4012.
[11] F. Gautschi and K. Bloch, *J. Biol. Chem.*, 1958, **233**, 1343.
[12] G. Popjak, DeW. S. Goodman, J. W. Cornforth, R. H. Cornforth, and R. Ryhage, *J. Biol. Chem.*, 1961, **236**, 1934.
[13] J. W. Cornforth, R. H. Cornforth, G. Popjak, and L. Yengoyan, *J. Biol. Chem.*, 1966, **241**, 3970.