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STEREOCHEMISTRY OF TRITIUM AT C-1 AND C-7 IN CHOLESTEROL DERIVED FROM (3R,2R)-2T-MEVALONIC ACID

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Consideration of the incorporation of mevalonic acid stereospecifically labeled with isotopic hydrogen at C-2, into squalene[1] and the cyclisation of the latter into sterols allows the prediction of the stereochemistry of the tritium atoms in the biosynthesised products. Hence, (3R,2R)-2T-mevalonic acid (I) leads to 1, 5R, 9R, 16R, 20R, 24 T_6-squalene (II). Cyclisation of this squalene will give T_6-lanosterol (III) labeled in the 1β, 7α, 15β, 22R, 30 or 31 and 26 or 27 positions. The transformation from lanosterol to cholesterol involves the intermediacy[2] first of a C-7 olefin (IVa) and then a 5, 7 diene (IVb). Consequently, one of the C-7 protons which originate from C-2 of mevalonic acid will be lost during the transformation[3]. In this communication we concern ourselves with the fate and the stereochemistry of the tritium atoms at C-1 and C-7 in cholesterol derived from (3R,2R)-2T-mevalonic acid.

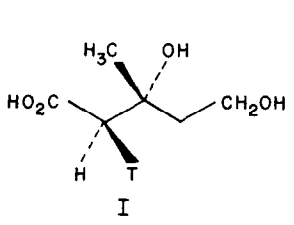
Cholesterol was biosynthesised[4] by incubation of (3R,2R)-2T-2^{14}C-mevalonic acid with a rat liver preparation[5]. The cholesterol was purified by thin layer chromatography in two systems (a) ethyl acetate: hexane 3:7 and (b) methanol:benzene 1:19. A portion of the material (1.72 x 10^6 d.p.m. ^{14}C)[6] was diluted with non-radioactive cholesterol (1.5 g), and crystallized to constant specific activity giving a T/^{14}C ratio of 10.1.

TABLE

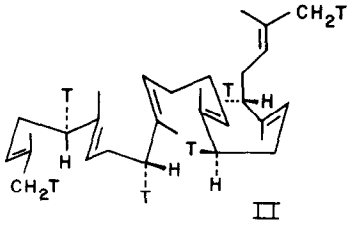
$T/^{14}C$ Ratios* And Number of Tritium Atoms

	$T/^{14}C$	No. T. Atoms
Cholesterol	10.1	5.00
Cholest-4-en-3-one (V)	9.7	4.80
5α-Cholestan-3-one (VI)	9.7	4.80
5α-Cholest-1-en-3-one (VII)	10.0	4.95
1,2-seco-5α-cholestan-1,3-dioic acid (VIII)	8.2	4.06
5α-Hydroperoxycholest-6-en-3β-ol (IX)	9.8	4.85
7α-Hydroperoxycholest-5-en-3β-ol (X)	9.7	4.80
Cholest-5-en-3β-ol-7-one	8.1	4.01

*The $T/^{14}C$ ratios were determined on recrystallized materials and were constant. The results represent the averages of nine determinations. The number of T atoms is calculated on the basis of the $T/^{14}C$ (10.1) ratio for cholesterol.



I



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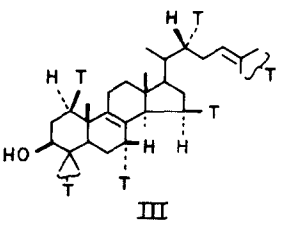

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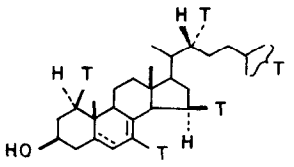
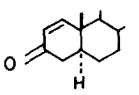
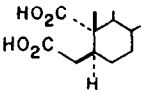


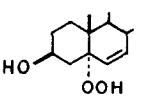
IV a, Δ^{7}
b, $\Delta^{5,7}$



VII



VIII



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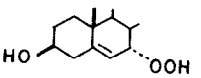
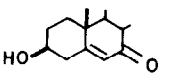


X



XI

For the determination of the presence and stereochemistry of tritium at C-1, the cholesterol was oxidised by the Oppenauer method[7] to cholest-4-en-3-one (V) ($T/^{14}C$ ratio 9.7). (Table). Reduction of V with lithium in liquid ammonia[8] gave 5α-cholestan-3-one (VI) ($T/^{14}C$ ratio 9.7). Dehydrogenation of VI with dichlorodicyanobenzoquinone (DDQ)[9] yielded 5α-cholest-1-en-3-one (VII) m.p. 109-110° ($T/^{14}C$ ratio 10.0). Finally, oxidative cleavage of VII gave 1,2-seco-5α-cholestan-1,3-dioic acid (VIII) m.p. 216.5-218.5° (m/e:420,M⁺): ($T/^{14}C$ ratio 8.2). The 1/5 decrease in $T/^{14}C$ ratio reflects the loss of one out of five tritium atoms during the oxidation, this tritium being located at C-1. Since Ringold and Turner[9] demonstrated that DDQ dehydrogenation of steroidal 5α-3-ketones proceeds with loss of the 1α and 2β axial hydrogens, then the tritium atom at C-1 must have the expected 1β-stereo-chemistry.

The demonstrated presence of five tritium atoms in the cholesterol indicates that isotopic hydrogen is still present at C-7. It could therefore be inferred that the formation of the Δ^7 intermediate (IVa) involves the elimination[10] of the 7β rather than the 7α hydrogen. Confirmation of the presence of a tritium atom at C-7 and of its stereochemistry was sought and is now described.

Cholesterol was photolysed in the presence of oxygen and hematoporphyrin[11] giving 5α-hydroperoxycholest-6-en-3β-ol (IX) m.p. 147-149° ($T/^{14}C$ ratio 9.8). Isomerisation[12,13] of IX gave 7α-hydroperoxycholest-5-en-3β-ol (X) m.p. 154-156° ($T/^{14}C$ ratio 9.7). Simultaneous isomerisation and dehydration of IX with cupric chloride[12] in pyridine gave cholest-5-en-3β-ol-7-one (XI) ($T/^{14}C$ ratio 8.1). The 1/5 decrease in $T/^{14}C$ ratio again indicates the loss of one out of five isotopic hydrogens and proves unequivocally the presence of a tritium atom at C-7. Since the photoxidation of cholesterol is a stereo-specific process involving the loss of the 7α hydrogen atom[11], the tritium must have the 7β configuration. It follows that the saturation of Δ^7 double bond of the 5,7-diene (IVb) involves the addition, at C-7, of a hydrogen which assumes the α-stereo-chemistry[14]. The overall result of the events occurring at C-7 during the conversion of lanosterol to cholesterol is therefore the inversion from the 7α to the 7β configuration of the proton originating from the pro-2R hydrogen of mevalonic acid.

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