The Formation of an 8,14-Diene System during Cholesterol Biosynthesis

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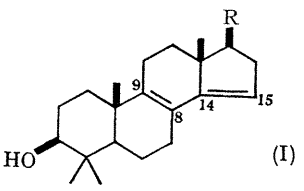
IT has been suggested that the removal of the 14-methyl group in sterol biosynthesis may occur through the oxidation of the C-15 position, involving the intermediacy of an 8,14-diene system.[1-3] The efficient conversion of both cholesta-8,14-dien-3β-ol,[1,3,4] and 4,4-dimethylcholesta-8,14-dien-3β-ol[2,3] into cholesterol has been demonstrated. We now demonstrate the formation of 4,4-dimethylcholesta-8,14-dien-3β-ol (I) from dihydrolanosterol under the conditions of cholesterol biosynthesis.

Dihydrolanosterol labelled with tritium in the 3α-position† (100 μg.; $1{\cdot}7 \times 10^6$ counts/min.) was incubated under aerobic conditions with a 10,000 *g* supernatant of rat liver homogenate[1,2] in the presence of a trap of 4,4-dimethylcholesta-8,14-dien-3β-ol (3 mg.) After 1 hr. the incubation was terminated, and the mixture of sterols was isolated and acetylated. A portion of the acetylated mixture containing 60% of the recovered radioactivity was then diluted with nonradioactive 4,4-dimethylcholesta-8,14-dien-3β-ol acetate (40 mg.) and applied to a thick layer silver nitrate HF_{254} plate which was developed in 70 : 30 benzene–petrol (b.p. 60—80°). After elution of the diene band and evaporation of the eluate to dryness, the product was spotted on a second plate and re-run in the same solvent system. The diene band was removed as before and recrystallized five times from diethyl ether–methanol to give the following radioactivities: 1st recrystallization: 2684; 2nd: 2659; 3rd: 2627; 4th: 2659; and 5th: 2653 c.p.m./mg. When a sample of the five-times-recrystallized diene was applied to an analytical silver nitrate thin-layer plate the entire radioactivity remained confined to the 4,4-dimethylcholesta-8,14-dien-3β-ol acetate region (R_F 0·41).‡ This represents a 10% overall incorporation of radioactivity from dihydrolanosterol into the trap. In a related control experiment carried out at the same time under anaerobic conditions, no radioactivity was detected in the 4,4-dimethylcholesta-8,14-dien-3β-ol acetate region. Under the general conditions of the above incubation [2-$^3H_2$]4,4-dimethylcholesta-8,14-dien-3β-ol (I) was converted into cholesterol in the presence of oxygen giving a 10—16% overall yield.§ For comparison it was shown that in parallel experiments lanosterol was converted into cholesterol giving a 15—20% yield.§

The cumulative evidence presented above allows us to conclude that a 8,14-diene system of the type (I) is involved in the conversion of the lanosterol nucleus into cholesterol. The sequence of reactions involved in the formation of the 8,14-diene from a 14-methyl precursor are discussed elsewhere.[1,2]



(I)

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† Dihydrolanosterol labelled at the 3α-position was specially chosen because during its biological conversion into cholesterol all compounds involving demethylation at C-4 will be rendered nonradioactive. This would thus facilitate the isolation of compounds which contain the 4,4-dimethyl group. The use of dihydrolanosterol instead of the natural precursor lanosterol is permissible because it is well known that the enzymes participating in reactions involving the steroid nucleus are relatively insensitive to the saturation status of the side chain.[2]

‡ The R_F values of several related compounds under the above chromatographic conditions are as follows: lanosterol acetate, 0·55; dihydrolanosterol acetate, 0·61; 4,4-dimethylcholest-5-en-3β-ol acetate 0·58; 4,4-dimethylcholest-8-en-3β-ol acetate 0·54; 4,4-dimethylcholesta-5,7-dien-3β-ol acetate 0·46; 4,4-dimethylcholesta-6,8(14)-dien-3β-ol acetate 0·53.

§ In all experiments described above, 100 μg. of the substrate was incubated with 10 ml. of the homogenate.[2]

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[4] B. N. Lutsky and G. J. Schroepfer, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 492.