ENZYMATIC CONVERSION OF $\Delta^{8,14}$-CHOLESTADIEN-3β-OL TO CHOLESTEROL[1]

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The nature of the intermediates in the enzymatic formation of cholesterol has been studied in several laboratories. Previous publications, reviewed elsewhere (Frantz and Schroepfer, 1967), have implicated as probable intermediates a number of sterols with nuclear double bonds in the following positions: Δ^{8}-, Δ^{7}-, $\Delta^{5,7}$-, and Δ^{5}. Considerations of the mechanism of the enzymatic removal of the methyl group attached to carbon atom 14 of lanosterol have led two laboratories to investigate the possible intermediary role of $\Delta^{8(14)}$-sterols in the biosynthesis of cholesterol. Fried, Dudowitz, and Brown (1968) have shown that 4,4-dimethyl-$\Delta^{8(14)}$-cholesten-3β-ol is convertible to cholesterol in rat liver homogenate preparations. In studies with similar preparations of rat liver in this laboratory (Lee and Schroepfer, 1968) the facile conversion of $\Delta^{8(14)}$-cholesten-3β-ol has also been demonstrated. A further finding of significance in consideration of the mechanism of the enzymatic removal of the methyl group attached to carbon atom 14

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of lanosterol is the recent observation by Canonica et al. (1968) that the 15β-hydrogen of lanosterol is lost upon enzymatic conversion to cholesterol.

As an extension of our previous work and clearly stimulated by this important observation of Canonica et al. we have prepared $\Delta^{8,14}$-cholestadien-3β-ol labeled with isotopic hydrogen to ascertain its convertibility to cholesterol by rat liver homogenate preparations. We wish to report the very efficient enzymatic conversion of this sterol to cholesterol.

Synthesis of $\Delta^{8,14}$-cholestadien-3-one

Authenticity and purity of the following intermediates were determined by these techniques: melting point, ultraviolet spectroscopy, infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectroscopy, elemental analysis, thin-layer chromatographic analysis on silica gel G plates and on plates of neutral alumina impregnated with silver nitrate (Kammereck, Lee, Paliokas and Schroepfer, 1967), and gas-liquid chromatographic analysis (3% QF-1 on gas-chrom Q).

$\Delta^{8,14}$-cholestadienyl-3β-acetate (m.p. 99.5 - 101.5°; λ^{EtOH} 250 mμ (ε 18,100))was prepared from $\Delta^{5,7}$-cholestadien-3β-ol (m.p. 151-152°) by treatment with a mixture of acetic acid, acetic anhydride, and hydrochloric acid (Fieser and Ourisson, 1953). $\Delta^{8,14}$-cholestadien-3β-ol was prepared from the acetate by heating under reflux with 15% methanolic potassium hydroxide. The free sterol crystallized from acetone in elongated prisms, m.p. 113.5 - 114.5°; λ^{EtOH} 250 mμ (ε 18,000). $\Delta^{8,14}$-cholestadien-3-one was prepared from the alcohol by oxidation with chromium trioxide in pyridine at 4°. The ketone was purified by column chromatography on alumina, and crystallization

from methanol-ether yielding short, compact needles, m.p. 137 - 138.5°; λ^{EtOH} 250 mμ (ε 18,200).

Synthesis of [3α - ^3H] - $\Delta^{8,14}$-cholestadien-3β-ol

[3α - ^3H] - $\Delta^{8,14}$-cholestadien-3β-ol (m.p. 113.5 - 114.2°; λ^{EtOH} 250 mμ (ε 18,100); single component on thin-layer and gas-liquid chromatographic analyses) was prepared from $\Delta^{8,14}$-cholestadien-3-one by reduction with tritium-labeled lithium aluminum hydride and purified by (1) chromatography on an alumina-silver nitrate column (Paliokas, Lee, and Schroepfer, 1968), (2) digitonide formation, and (3) recrystallization from methanol. The specific activity was 39.9 mc/mmole. The radiopurity was judged to be in excess of 96% on the basis of analysis by thin-layer chromatography on plates of silica gel G and by gas-liquid chromatography.

Conversion of [3α - ^3H] - $\Delta^{8,14}$-cholestadien-3β-ol to cholesterol by rat liver homogenate preparations.

[3α - ^3H] - $\Delta^{8,14}$-cholestadien-3β-ol (148.5 μg; 2.1 x 10^7 cpm) in propylene glycol (100 μl) was incubated with 30 ml of a 10,000 x g supernatant fraction of a rat liver homogenate for 3 hours in air at 37° (Paliokas and Schroepfer, 1968). The sterols were isolated from the saponified incubation medium by extraction with petroleum ether (88% recovery of the incubated radioactivity) and they were subjected to chromatography on an alumina-silver nitrate column (1 x 34 cm) using a mixture of chloroform and acetone (98:2) as the eluting solvent. The resulting chromatogram is shown in Figure 1. Approximately 75% of the recovered radioactivity was associated chromatographically with cholesterol. The labeled cholesterol in fractions 62-75 was further characterized as such by purification by way of the dibromide

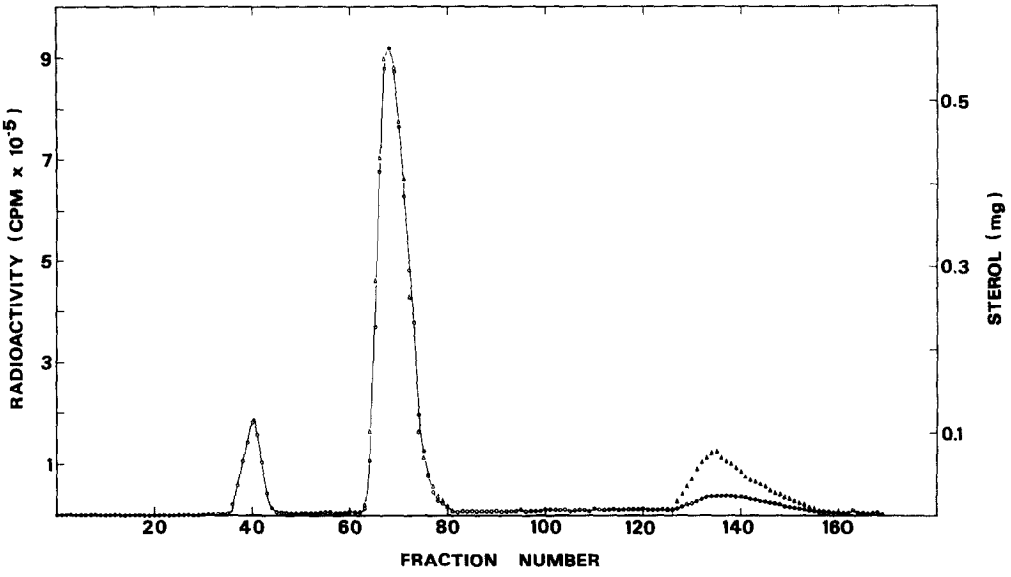

Figure 1. *Column chromatogram showing enzymatic conversion of [3α - ^3H] - $\Delta^{8,14}$-cholesten-3β-ol to cholesterol. o——o, radioactivity; Δ——Δ, cholesterol measured colorimetrically; ▲——▲, $\Delta^{8,14}$-cholestadien-3β-ol measured colorimetrically.*

(Paliokas and Schroepfer, 1968) after dilution with unlabeled cholesterol. The specific activities before and after this purification were 67,000 and 67,800 cpm/mg, respectively. A significant amount of the radioactivity (10% of the recovered tritium) emerged from the column in a less polar peak (fractions 35-45). The precise nature of this material has not been determined. C_{27}-sterols with similar mobility include Δ^8-cholesten-3β-ol, $\Delta^{8(14)}$-cholesten-3β-ol, Δ^7-cholesten-3β-ol, and cholestan-3β-ol.

These studies establish that $\Delta^{8,14}$-cholestadien-3β-ol is convertible to cholesterol by rat liver homogenate preparations. Boiled enzyme controls were negative. The efficient nature of the observed conversion suggests a possible intermediary role of this compound in the biosynthesis of cholesterol.

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