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

Wen-Hui Lee and G. J. Schroepfer, Jr.

Division of Biochemistry, Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois 61801

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The enzymatic conversion of lanosterol to cholesterol involves three general processes: reduction of the Δ^{24}-double bond, "shift" of the nuclear double bond from the Δ^{8}-position to the Δ^{5}-position, and removal of the three "extra" methyl groups (Frantz and Schroepfer, 1967). The removal of each of the "extra" methyl groups (30, 31, and 32) of lanosterol have been considered to proceed via an initial oxygen-dependent hydroxylation followed by dehydrogenations to yield the corresponding aldehydes and carboxylic acid (Pudles and Bloch, 1960). The decarboxylation of sterols with carboxylic functions on carbon atom 4 would be greatly facilitated by the presence of a ketone function at carbon atom 3. Indeed, Lindberg, Gautschi and Bloch (1963) and Swindell and Gaylor (1967) have presented several types of experimental evidence compatible with an intermediary (not necessarily obligatory) role for 3-ketosterols in the removal of the "extra" methyl groups at carbon atom 4 of lanosterol and related sterols. In the case of the removal of the methyl group (carbon atom 32) at carbon atom 14 of cholesterol precursors with a Δ^{8} (or Δ^{7}) nuclear double bond, hydroxylation and successive dehydrogenations would yield a β,γ-unsaturated acid. After reviewing information available concerning the mechanism of non-enzymatic decarboxylations of β,γ-unsaturated acids (Arnold, Elmer, and

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Dodson, 1950; Bigley and Thurman, 1967) and stimulated by a discussion by Richards and Hendrickson (1964), we noted that an analogous decarboxylation of a Δ^{8}-32-steroidal acid would yield a $\Delta^{8(14)}$-sterol. The convertability of $\Delta^{8(14)}$-sterols to cholesterol has not been reported previously. Accordingly, we have prepared [3α-^{3}H]-$\Delta^{8(14)}$-cholesten-3β-ol by chemical synthesis and tested its convertability to cholesterol by rat liver homogenate preparations.

Synthesis of [3α-^{3}H]-$\Delta^{8(14)}$-cholesten-3β-ol

$\Delta^{8(14)}$-cholesten-3β-ol was prepared from $\Delta^{5,7}$-cholestadien-3β-ol (m.p. 151-152°) by catalytic reduction in ethyl acetate: acetic acid (95:5) using a platinum oxide catalyst. The product was characterized by melting point (120-121°), optical rotation ($[\alpha]_D^{20}$ + 23.4°, c. 1.28)[2], infrared spectroscopy, 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 by gas-liquid chromatographic analysis according to Clayton (1962). $\Delta^{8(14)}$-cholesten-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 by crystallization from methanol-water (m.p. 101-102°; $[\alpha]_D^{20}$ + 39°; single component on thin-layer and gas-liquid chromatographic analysis). The ketone was reduced with lithium aluminum tritide yielding, after purification on an alumina-$AgNO_3$ column (Paliokas, Lee and Schroepfer, 1968) and recrystallization from methanol, [3α-^{3}H]-Δ^{8}-cholesten-3β-ol (specific activity 3.11 x 10^{7} dpm per mg) which was characterized by melting point (118.5-120°), optical rotation ($[\alpha]_D^{20}$ + 24.1°, c 2.35), infrared spectroscopy and mass spectrometry. The radiopurity was judged to be in excess of 98% on the basis of analysis by thin-layer chromatography on two types of support, by gas-liquid chromatography, and by column chromatography of the acetate

[2] Optical rotations were measured in chloroform.

derivative on columns of alumina impregnated with silver nitrate.

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

[3α-^{3}H]-$\Delta^{8(14)}$-cholesten-3β-ol (37.3 μg; 685,600 cpm) in propylene glycol (100 μl) was incubated with a 10,000 x g supernatant fraction (97 ml) for 3 hours in air as described previously (Paliokas and Schroepfer, 1968). The sterols were isolated from the saponified incubation medium by extraction with petroleum ether (88% recovery of the incubated radioactivity) A portion of recovered sterol mixture was applied to an alumina-silver nitrate column (100 x 1 cm; Paliokas, Lee and Schroepfer, 1968). Chloroform: acetone (98:2) was used as the eluting solvent. The resulting chromatogram is shown in Figure 1. Approximately 33% of the radioactivity was associated chromatographically with cholesterol. The labeled material corresponding to fractions 50 through 63 was pooled, diluted with carrier cholesterol, and purified by way of the dibromide (Paliokas and Schroepfer, 1968). The specific activities before and after this purification were 2980 and 2930

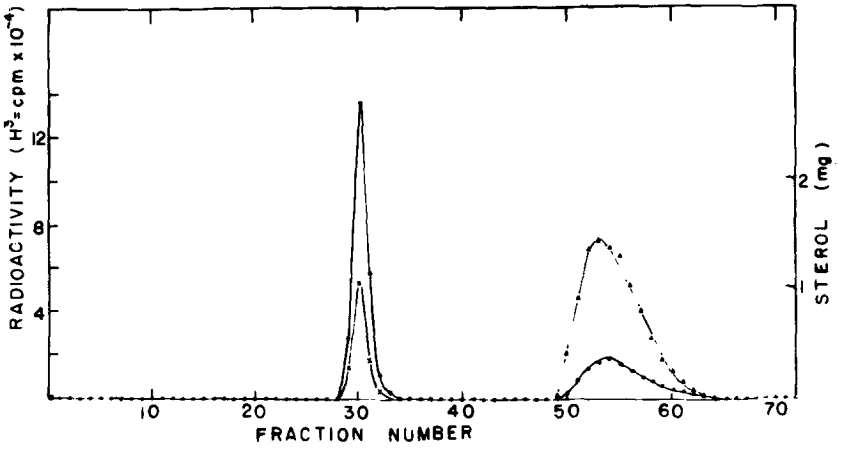

Figure 1. Column chromatogram showing enzymatic conversion of [3α-^{3}H]-$\Delta^{8(14)}$-cholesten-3β-ol to cholesterol. O—O, radioactivity; x—x, carrier $\Delta^{8(14)}$-cholesten-3β-ol, measured colorimetrically; ▲—▲, cholesterol, measured colorimetrically.

cpm/mg, respectively. Boiled enzyme controls were negative. Incubation carried out in an atmosphere of helium resulted in virtually quantitative recovery (>98%) of unchanged substrate. Under similar conditions, very efficient conversion (85 to 95%) of Δ^8-cholesten-3β-ol to Δ^7-cholesten-3β-ol has routinely been observed. If Δ^7-cholesten-3β-ol is an intermediate in the overall conversion of $\Delta^{8(14)}$-cholesten-3β-ol to cholesterol, it appears that molecular oxygen is required for the conversion of $\Delta^{8(14)}$-cholesten-3β-ol to Δ^7-cholesten-3β-ol. Further studies of the metabolism of $\Delta^{8(14)}$-cholesten-3β-ol in animal tissues and an attempt to isolate $\Delta^{8(14)}$-cholesten-3β-ol from animal tissue are in progress.

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