ENZYMATIC CONVERSION OF CHOLEST-8(14)-EN-3β,15α-DIOL AND CHOLEST-8(14)-EN-3β,15β-DIOL TO CHOLESTEROL*

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Summary: Reduction of 3β-benzoyloxy-cholest-8(14)-en-15-one with lithium aluminum hydride yielded two epimers (at C-15) of cholest-8(14)-en-3β,15-diol. Reduction with lithium aluminum tritide yielded [15α-^{3}H]-cholest-8(14)-en-3β,15β-diol and [15β-^{3}H]-cholest-8(14)-en-3β,15α-diol. Both labeled compounds served as efficient substrates for cholesterol formation in rat liver homogenate preparations.

The results of recent studies have indicated the convertibility of cholest-8(14)-en-3β-ol (1,2) and 4,4-dimethyl-cholest-8(14)-en-3β-ol (3,4) to cholesterol in rat liver homogenate preparations. Moreover, evidence indicating the presence of cholest-8(14)-en-3β-ol in rat skin has been presented (2,5). The mechanisms involved in the conversion of the $\Delta^{8(14)}$-sterols to cholesterol are incompletely understood at this time. The convertibility of cholest-8(14)-en-3β-ol to cholest-7-en-3β-ol in rat liver homogenate preparations under aerobic conditions has been demonstrated (2). While the efficient conversion of cholest-8-en-3β-ol to cholest-7-en-3β-ol can readily be demonstrated under anaerobic conditions (6,7), no detectable conversion of cholest-8(14)-en-3β-ol to cholest-8-en-3β-ol or cholest-7-en-3β-ol could be demonstrated (2). This combination of findings suggested the presence of an oxygen-dependent step in the conversion of cholest-8(14)-en-3β-ol to cholest-7-en-3β-ol. In an attempt to clarify this problem we

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have prepared, by chemical synthesis, [15α-^{3}H]-cholest-8(14)-en-3β,15β-diol and [15β-^{3}H]-cholest-8(14)-en-3β,15α-diol and studied their convertibility to cholesterol in rat liver homogenate preparations. Both compounds were found to be efficiently converted to cholesterol.

Synthesis of cholest-8(14)-en-3β,15-diols

3β-Benzoyloxy-cholest-8(14)-en-15-one, (m.p. 155-156°) [literature: 156° (8); 156-158° (9)] was prepared according to Knight et al. (9). λ_{max} 258 mμ (ε 15,650) [literature: λ_{max} 258 mμ (ε 15,800) (8)]. The infrared spectrum, elemental analysis, and mass spectrum were compatible with the assigned structure. The compound showed a single component on thin-layer chromatographic analysis in three different systems.

3β-Benzoyloxy-cholest-8(14)-en-15-one (3.97 mmole) in ether (104 ml) was reduced with lithium aluminum hydride (16 mmole) for two hours at room temperature. The excess reagent was decomposed by the successive addition of ethyl acetate, a saturated solution of ammonium chloride, and water. Extraction with ether yielded 2.6 g of material which was subjected to chromatography on an activated silicic acid column (75 x 3 cm). Using benzene-ether (90:10) as the eluting solvent, fractions 25 ml in volume were collected. One of the epimeric diols (672 mg; designated diol A), eluted in fractions 250 through 375, was recrystallized four times from acetone-water. The resulting crystals melted at 174.0-175.5°. C 80.5, H 11.4 (calculated for $C_{27}H_{46}O_2$: C 80.5, H 11.5). The mass spectrum showed a molecular ion at m/e 402. The NMR spectrum (100 MHz) showed no absorption due to an olefinic proton. The infrared spectrum showed a broad absorption at 3,290 cm^{-1} (OH). One component was observed upon thin-layer chromatographic analysis in three different systems. The second epimeric diol (930 mg; designated diol B), eluted in fractions 425 through 650, was recrystallized three times from acetone-water. The crystalline product melted at 137.0-137.5°. C 80.5, H 11.5 (calculated for $C_{27}H_{46}O_2$: C 80.5, H 11.5). The mass spectrum showed a molecular ion at m/e 402. The NMR spectrum showed no absorption

due to an olefinic proton. The infrared spectrum showed a broad absorption band at 3,490 cm^{-1} (OH). The compound showed a single component upon thin-layer chromatographic analysis in three different systems.

Synthesis of [15α-^3H]-cholest-8(14)-en-3β,15β-diol and [15β-^3H]-cholest-8(14)-en-3β,15α-diol

3β-Benzoyloxy-cholest-8(14)-en-15-one (200 mg) in ether (20 ml) was reduced with [^3H]-lithium aluminum hydride (20 mCi; 11.8 mg) at room temperature for 45 minutes. Unlabeled lithium aluminum hydride (25 mg) was added and the mixture was stirred for an additional 45 minutes. The labeled sterols were recovered from the reaction mixture as described above and subjected to chromatography on an activated silicic acid column (30 x 1.5 cm). Using benzene-ether (90:10) as the eluting solvent, fractions 25 ml in volume were collected. [^3H]-Diol A (∿ 15 mg) was eluted in fractions 16 through 24, and,after recrystallization from acetone-water, melted at 175-176°. The specific activity was ∿ 1.1 x 10^5 cpm per μg. [^3H]-Diol B (131 mg) was eluted in fractions 28 through 54 and, after recrystallization from acetone-water, melted at 136-137°. The specific activity was ∿1.4 x 10^5 cpm per μg. The identity of the [^3H]-diols was further confirmed by cochromatography of the labeled diols with authentic unlabeled diol A and diol B on silicic acid columns. The sample of [^3H]-diol B used in the studies described below contained ∿ 2.6% diol A.

Enzymatic Conversion of [^3H]-Diol A to Cholesterol

[^3H]-Diol A (4.6 x 10^6 cpm) in propylene glycol (0.1 ml) was incubated with a 10,000 x g supernatant fraction (50 ml) of a rat liver homogenate (10) for 3 hours at 37°. Extraction of the sterols from the saponified incubation mixture with petroleum ether yielded 2.25 x 10^6 cpm. A portion of this material was applied to a silicic acid-Super Cel column (10) (50 x 1 cm). Using benzene as the eluting solvent, fractions 4 ml in volume were collected. Approximately 95.5% of the radioactivity (88% recovery)

was eluted in fractions 12 through 15, corresponding to the location of authentic unlabeled cholesterol. The contents of these fractions were pooled and subjected to chromatography on an alumina-Super Cel-silver nitrate column (10) (50 x 1 cm). Using chloroform-acetone (97:3) as the eluting solvent, fractions 4 ml in volume were collected. A single radioactive component was observed which corresponded in mobility to that of authentic cholesterol. A portion of this material was diluted with unlabeled cholesterol and purified by way of the dibromide. The specific activity of the [^{3}H]-cholesterol before and after this purification was 56 cpm per μg and 54 cpm per μg, respectively.

Enzymatic Conversion of [^{3}H]-Diol B to Cholesterol

[^{3}H]-Diol B (6.0 x 10^6 cpm) was incubated with a 10,000 x g supernatant fraction of a rat liver homogenate and the labeled sterols (5.5 x 10^6 cpm) were recovered from the saponified incubation mixture as described above. A portion of this material was applied to a silicic acid-Super Cel column (10) (50 x 1 cm) and the column was eluted with benzene as described above. Approximately 98.6% of the radioactivity (100% recovery) was eluted in fractions 18 through 27, corresponding to the location of authentic cholesterol. The contents of these fractions were pooled and subjected to chromatography on an alumina-Super Cel-silver nitrate column (10) (50 x 1 cm). Using chloroform-acetone (97:3) as the eluting solvent, fractions 4 ml in volume were collected. Approximately 90% of the recovered radioactivity was eluted in fractions 29 through 41, corresponding to the location of carrier cholesterol. A portion of this material was diluted with unlabeled cholesterol and purified by way of the dibromide. The specific activity before and after this purification was 45.7 cpm per μg and 45.5 cpm per μg, respectively.

Discussion

Evidence has been presented which indicates that both epimers (at

C-15) of cholest-8(14)-en-3β,15-diol serve as efficient precursors of cholesterol in rat liver homogenate preparations. This result differs from that recently observed with the epimers (at C-15) of 14α-methyl-cholest-7-en-3β,15-diol. In the latter case, only one of the two epimers served as a precursor of cholesterol (11). The finding that 15-hydroxy derivatives of cholest-8(14)-en-3β-ol are convertible to cholesterol may provide an explanation for the apparent requirement for molecular oxygen in the overall conversion of cholest-8(14)-en-3β-ol to cholest-7-en-3β-ol and suggests the possibility of a reaction sequence such as the following: cholest-8(14)-en-3β-ol ⟶ cholest-8(14)-en-3β,15-diol ⟶ cholesta-7,14-dien-3β-ol ⟶ cholest-7-en-3β-ol. Further studies of the metabolism of the two epimeric 15-hydroxy derivatives of cholest-8(14)-en-3β-ol are in progress.

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