CHEMICAL SYNTHESIS OF CHOLESTA-5,7,24-TRIEN-3β-OL AND DEMONSTRATION OF ITS CONVERSION TO CHOLESTEROL IN THE RAT*

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Introduction--Cholesta-5,7,24-trien-3β-ol (VI in Fig. 1) was first proposed as a possible intermediate in cholesterol biosynthesis by Johnston and Bloch (1957). A sterol with the probable structure of (VI) has been detected by Frantz et al. (1962), using MER-29 treated guinea pigs, and by Dvornik et al. (1964), using a pig treated with both MER-29 and AY-9944. This communication describes the first chemical synthesis of cholesta-5,7,24-trien-3β-ol, as well as the first clearly documented demonstration of the conversion of this sterol to cholesterol in both the intact rat and a cell-free preparation of rat liver.

Chemical Synthesis--(see Fig. 1) 1.6 g of cholesta-5,24-dien-3β-ol[1] (I) was converted by Oppenauer oxidation to cholesta-4,24-dien-3-one (II), yield 1.2 g, m.p. 83-85°; UV, $\lambda_{max}^{ethanol}$ 240 mμ (ε 17,400); $[\alpha]_D^{25}$ + 85°; calculated for $C_{27}H_{42}O$, C 84.75, H 11.09; analyzed, C 84.10, H 10.70. (II) when treated with ethyl orthoformate and p-toluenesulfonic acid gave

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[1] Purchased from Organon, Inc., West Orange, N.J.

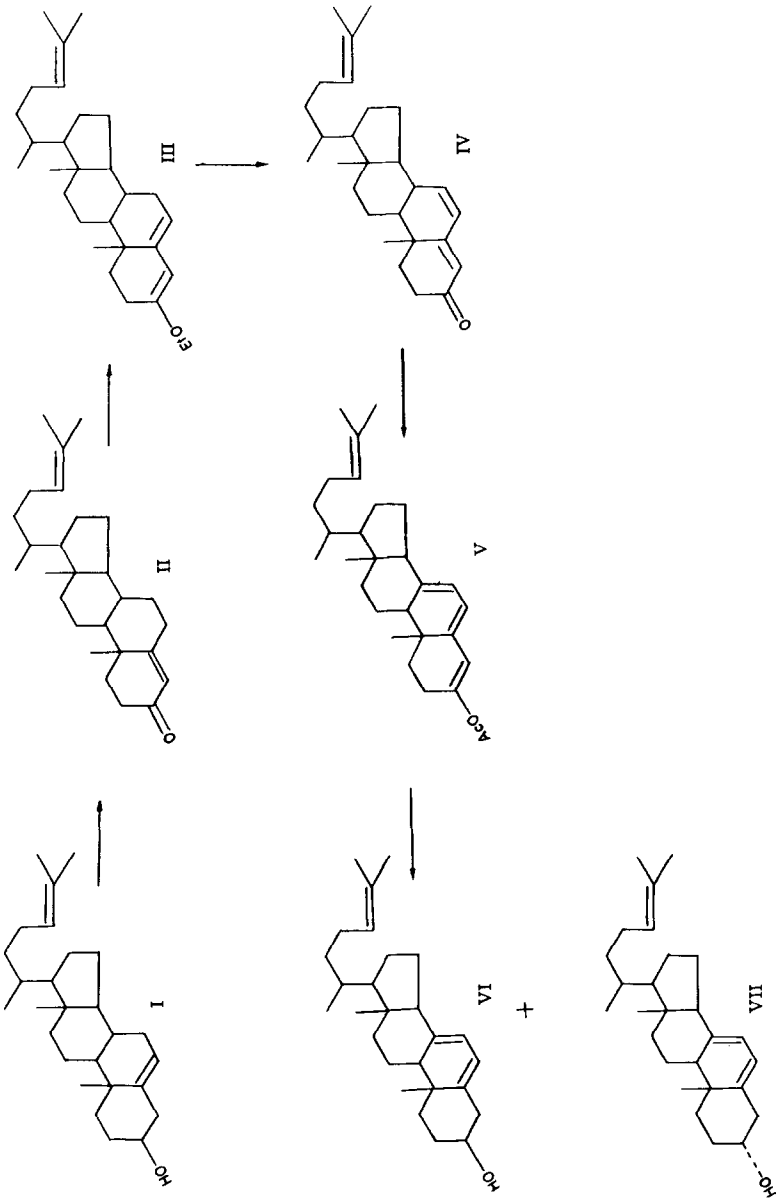

Figure 1. Chemical synthesis of cholesta-5,7,24-trien-3β-ol.

3-ethoxy-cholesta-3,5,24-trien (III), which when treated with activated manganese dioxide yielded 400 mg of cholesta-4,6,24-trien-3-one (IV), m.p. 98-99°; UV, $\lambda_{max}^{ethanol}$ 284 mμ (ε 26,000); $[\alpha]_D^{25}$ + 26°; calculated for $C_{27}H_{40}O$, C 85.2, H 10.6; analyzed, C 84.50, H 10.8. (IV) was converted to (VI) in a manner similar to that described in a synthesis of 7-dehydrocholesterol by Dauben et al. (1953). (IV) was treated with acetyl chloride and acetic anhydride yielding 3-acetoxy-cholesta-3,5,7,24-tetraen (V) which was treated with $NaBH_4$ in methanol-ether to yield crude cholesta-5,7,24-trien-3β-ol (VI). (VI) was purified by precipitation as the digitonide (Sperry and Webb, 1950), chromatography on silicic acid (Frantz, 1963), and crystallization from methanol, yield 20 mg, m.p. 102-102.5°; UV, $\lambda_{max}^{cyclohexane}$ 271 mμ (ε 10,700), 282 mμ (ε 11,300), 294 mμ (ε 6400); $[\alpha]_D^{25}$ -111°; n.m.r., τ 9.38 (C-18 methyl); 9.04 (C-19, C-21 methyls); 8.31, 8.37 (C-25 vinyl methyls); 5.37 (C-6, C-7, C-24 vinyl protons); calculated for $C_{27}H_{42}O$, C 84.75, H 11.09; analyzed, C 84.85, H 11.10.

Cholesta-5,7,24-trien-3β-ol-3α-^{3}H was prepared using the same synthetic method but with 50 mc of NaB^3H_4 being used in the conversion of (V) to (VI). The IR and UV spectra of radioactive (VI) were essentially identical with those obtained of unlabeled (VI), specific activity 1.167 X 10^4 cpm/μg (counter efficiency of 33%).

Biological Studies-In Vitro--A cell-free homogenate of rat liver was prepared as described by Frantz and Bucher (1954) using 0.1 M phosphate buffer, pH 7.23. The incubation flasks contained the following substances:

(1) 1.05 μg (12,220 cpm) of cholesta-5,7,24-trien-3β-ol-3α-^{3}H dissolved in 200 μl of propylene glycol.

(2) 6 mg TPNH

(3) 15 ml of cell-free homogenate

Incubation was carried out at 37°, under nitrogen for 3 hours in a Dubnoff shaker. The reaction was stopped by the addition of absolute ethanol and the sterols were extracted with petroleum ether. The solvent was removed

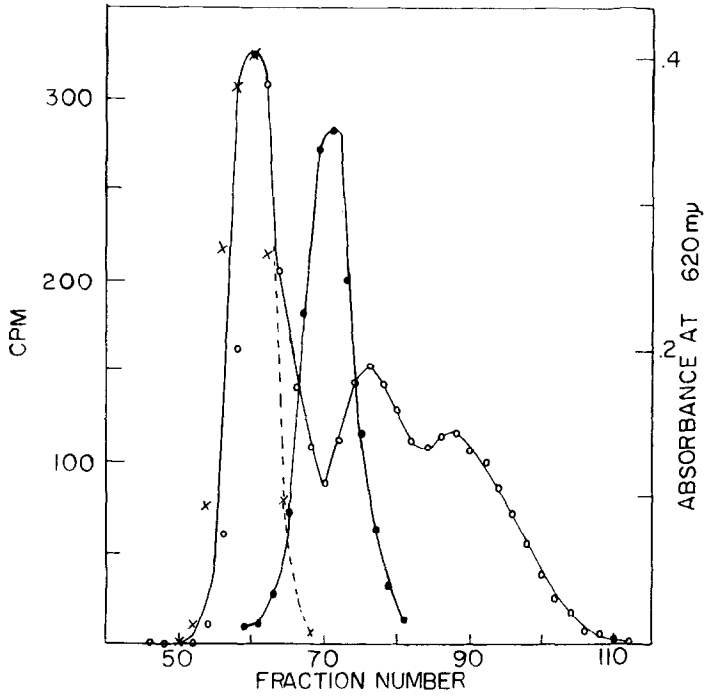

Figure 2. Chromatogram of the sterols obtained from a Bucher homogenate of rat liver which was incubated with cholesta-5,7,24-trien-3β-ol-3α-^{3}H.

o———o, radioactivity; x———x, cholesterol measured colorimetrically; •———•, 7-dehydrocholesterol measured colorimetrically.

under a stream of nitrogen, the residue dissolved in 4 ml of benzene, and the following carrier compounds added: (1) cholesterol, 5 mg (2) 7-dehydrocholesterol, 5.1 mg. This mixture was applied to a 2 x 110 cm silicic acid column with benzene as the eluting solvent. Figure 2 shows the chromatogram obtained. Tubes 54-68 contained the added carrier cholesterol; and, as can be seen, a peak of radioactivity (peak I) is closely coincident with cholesterol measured colorimetrically. Further proof that this peak represents primarily radioactive cholesterol synthesized by the homogenate was obtained by passage of odd-numbered tubes 53-69 thru the dibromide (Fieser, 1953c; Frantz et al., 1959). Specific activity obtained before the dibromide was 7.24 cpm/mg and after the dibromide 5.32 cpm/mg; therefore, 73.5% of the

radioactivity was retained. As can be seen from Figure 2, there is evidence of heterogeneity present on the more polar edge of the cholesterol peak (peak I). This impurity probably represents 7-dehydrocholesterol and/or desmosterol.

The identity of radioactive peak II (Tubes 70-84) is unknown; however, its slightly more polar chromatographic behavior (when compared with carrier 7-dehydrocholesterol) would be compatible with the chromatographic behavior of desmosterol. The third radioactive peak (peak at tube 88) corresponds in chromatographic position to that of the substrate, cholesta-5,7,24-trien-3β-ol-3α-^{3}H.

Biological Studies-In Vivo--Approximately 100,000 cpm (8.6 μg) of cholesta-5,7,24-trien-3β-ol-3α-^{3}H was dissolved in 0.05 ml of absolute ethanol; to this solution, 0.5 ml of 0.15 M sodium chloride was added. The resulting emulsion was injected into the portal vein of a female Sprague-Dawley rat which had been previously anesthetized with ether. The abdominal incision was closed and the rat placed in a cage with free access to food and water. Seventy-two hours later the rat was sacrificed, the liver was refluxed in 15% alcoholic-KOH (40 ml) for 4 hours, an equal volume of water was added, and the mixture extracted five times with 80 ml portions of petroleum ether. The solvent was removed under a stream of nitrogen, approximately 5 ml benzene added, and the solution was placed on a 2 X 110 cm column. The chromatogram obtained (Fig. 3) is very similar in appearance to that shown in Fig. 2. Tubes 64-80 contained a radioactive peak which coincided closely with cholesterol measured colorimetrically. This radioactive peak was further characterized as being primarily cholesterol by passage thru the dibromide (odd-numbered tubes 65-79). Specific activity before the dibromide was 7.38 cpm/mg; after the dibromide the specific activity was 5.74 cpm/mg. Thus 77.8% of the radioactivity was retained.

Discussion--Several investigators (Cook et al., 1954; Kandutsch 1961,

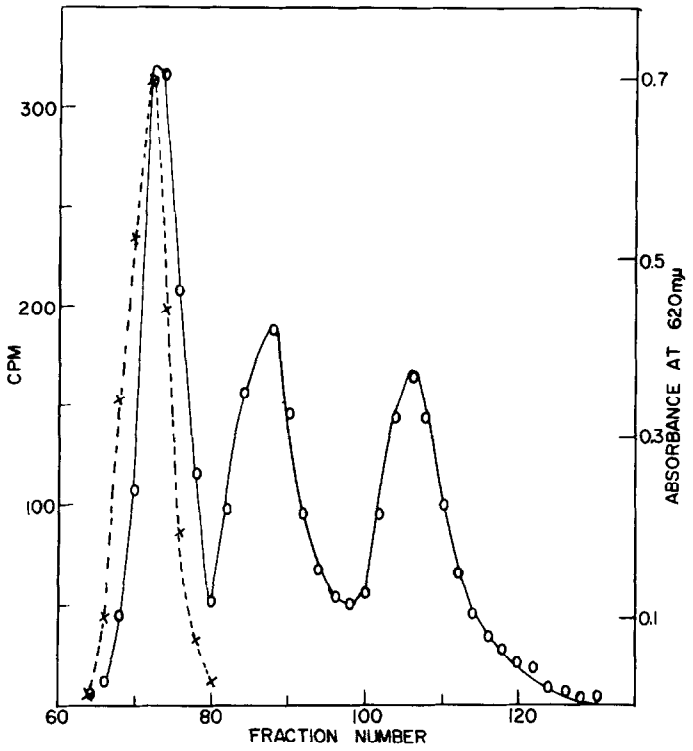

Figure 3. Chromatogram of the liver sterols of a rat 72 hours after the intraportal injection of cholesta-5,7,24-trien-3β-ol-3α-^{3}H.
x------x, cholesterol measured colorimetrically;
o———o, radioactivity

1962; Schroepfer and Frantz, 1961; Dvornik et al., 1963; Dempsey et al., 1964) have obtained evidence which supports an intermediary role for the $\Delta^{5,7}$ sterol, 7-dehydrocholesterol, in the biosynthesis of cholesterol. Also, the occurrence of Δ^{24}-sterols in the biosynthesis of cholesterol is well established (Clayton and Bloch, 1956; Johnston and Bloch, 1957; Alexander and Schwenk, 1957; Stokes et al., 1958; Clayton et al., 1963). These facts, coupled with the detection of a sterol with the probable structure of (VI) by Frantz et al. (1962) and by Dvornik et al. (1964), suggest that (VI) can occur

in biological systems.[2] Data presented in this communication demonstrates the biological conversion of cholesta-5,7,24-trien-3β-ol-3α-^{3}H to cholesterol both in vivo and in vitro. Thus the evidence cited above supports but does not necessarily prove that (VI) is an intermediate in the biosynthesis of cholesterol.

Summary--The chemical synthesis of cholesta-5,7,24-trien-3β-ol, a possible intermediate in cholesterol biosynthesis, is described. Also, conversion of cholesta-5,7,24-trien-3β-ol-3α-^{3}H to cholesterol in both the intact rat and a cell-free preparation of rat liver is described.

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REFERENCES

Alexander, G.J., and Schwenk, E., Arch. Biochem. Biophys., 66, 381 (1957).
Clayton, R.B., and Bloch, K., J. Biol. Chem., 218, 305 (1956).
Clayton, R.B., Nelson, A.N., and Frantz, I.D., Jr., J. Lipid Research, 4, 166 (1963).
Cook, R.P., Kliman, A., and Fieser, L.F., Arch. Biochem. Biophys., 52, 439 (1954).
Dauben, W.G., Eastham, J.F., Micheli, R.A., Takemura, K.H., Mandell, L., and Chemerda, J.M., J. Am. Chem. Soc., 75, 3255 (1953).
Dvornik, D., Kraml, M., Dubuc, J., Givner, M., and Gaudry, R., J. Am. Chem. Soc., 85, 3309 (1963).
Dvornik, D., Kraml, M., and Bagli, J.F., J. Am. Chem. Soc., 86, 2739 (1964).
Dempsey, M.E., Seaton, J.D., Schroepfer, G.J., Jr., and Trockman, R.W., J. Biol. Chem., 239, 1381 (1964).
Fieser, L.F., J. Am. Chem. Soc., 75, 5421 (1953).
Frantz, I.D., Jr., and Bucher, N.L.R., J. Biol. Chem., 206, 471 (1954).
Frantz, I.D., Jr., Davidson, A.G., Dulit, E., and Mobberley, M.L., J. Biol. Chem., 234, 2290 (1959).
Frantz, I.D., Jr., Sanghvi, A.T., and Clayton, R.B., J. Biol. Chem., 237, 3381 (1962).
Frantz, I.D., Jr., J. Lipid Research, 4, 176 (1963).
Johnston, J.D., and Bloch, K., J. Am. Chem. Soc., 79, 1145 (1957).
Kandutsch, A.A., Federation Proc., 20, 282 (1961).
Kandutsch, A.A., J. Biol. Chem., 237, 358 (1962).
Schroepfer, G.J., Jr., and Frantz, I.D., Jr., J. Biol. Chem., 236, 3127 (1961).
Sperry, W.M., and Webb, M., J. Biol. Chem., 187, 97 (1950).
Stokes, W.M., Hickey, F.C., and Fish, W.A., J. Biol. Chem., 232, 347 (1958).

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[2] Formation of cholesta-5,7,24-trien-3β-ol from either cholesta-7,24-dien-3β-ol-^{3}H or unlabeled cholesta-8,24-dien-3β-ol and the probable pathways of conversion of the trienol to cholesterol by a rat liver enzyme preparation has recently been demonstrated (Dempsey, M.E., J. Biol. Chem., in press).