Chemical Synthesis of $\Delta^{7,24}$-[3α-3H]Cholestadien-3β-ol and Its Conversion to Cholesterol in the Rat*

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SUMMARY

$\Delta^{7,24}$-[3α-3H]Cholestadien-3β-ol was synthesized by catalytic hydrogenation of $\Delta^{5,7,24}$-[3α-3H]cholestatrien-3β-ol. The elemental composition was proved correct by high resolution mass spectrometry. The mass spectrum of $\Delta^{7,24}$-[3α-3H]cholestadien-3β-ol was compared with the spectra of $\Delta^{5}$- and $\Delta^{7}$-cholesten-3β-ol and $\Delta^{5,24}$-cholestadien-3β-ol. The infrared spectrum of synthetic $\Delta^{7,24}$-[3α-3H]cholestadien-3β-ol is compatible with that of the biological sterol. Their metabolic behavior is also similar.

A sterol with the probable structure of $\Delta^{7,24}$-cholestadien-3β-ol has been isolated by Frantz and Mobberley (1) from the livers, and by Clayton et al. (2) and Frantz et al. (3) from the skins of MER-29-treated rats. The conversion of the biosynthetic $\Delta^{7,24}$-cholestadien-3β-ol to cholesterol and intermediates has been studied by Frantz et al. (3) in vivo and Dempsey (4) in vitro. This paper describes the chemical synthesis of $\Delta^{7,24}$-[3α-3H]cholestadien-3β-ol and its conversion to cholesterol and some intermediates in a cell-free preparation of rat liver.

MATERIALS AND METHODS

$\Delta^{5,24}$-Cholesterol-3β-ol (desmosterol), 90% pure, was obtained from Organon, Inc., West Orange, N.Y., tris(triphenylphosphine)rhodium chloride from K & K Laboratories, Inc., Plainview, N.Y., and sodium [αH]borohydride from International Chemical and Nuclear Corp., Irvine, Calif. Other chemicals were reagent grade. The solvents were redistilled before use.

The silicic acid-Super Cel chromatography has previously been described by Clayton et al. (2). The silver nitrate chromatography was done according to the method of Zacchei (5) or Paliokas et al. (6) and Lee et al. (7). Melting points were determined in sealed evacuated capillary tubes in a Thomas-Hoover melting point apparatus. Radioactivity was measured with a Packard Tri-Carb scintillation spectrometer. Ultraviolet spectra were recorded on a Beckman model DU spectrometer. Infrared spectra were recorded on a Perkin-Elmer 521 spectrometer equipped with a reflecting beam condenser unit. KBr pellets were made with a Perkin-Elmer micro disc using 1.5-mm discs. The mass spectra were run by Dr. William WAVELENGTH (MICRONS)

FIG. 1. Infrared spectra of $\Delta^{5}$-cholesten-3β-ol (A) and $\Delta^{7,24}$-cholestadien-3β-ol (B).

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L. Budde at Purdue University. The spectrum of $\Delta^{7,24}$-$[3\alpha^3H]$-cholestadien-3β-ol was recorded with a CEC 21-110 spectrometer, ionizing energy 70 e.v., ion current 100 μA, the spectra of the other sterols with a Hitachi RMV-6A spectrometer, ionizing energy 75 e.v., ion current 50 μA. The high resolution spectrum of $\Delta^{7,24}$-$[3\alpha^3H]$-cholestadien-3β-ol was recorded with a CEC-21-110-B spectrometer.

**EXPERIMENTAL PROCEDURE AND RESULTS**

*Chemical Synthesis—$\Delta^{5,7,24}$-$[3\alpha^3H]$-cholestatrien-3β-ol was synthesized according to the procedure of Scallen (8). For additional purification the sterol was acetylated and the acetate, 20 mg, chromatographed on a silver nitrate column (1 × 45 cm) (5). Small amounts of impurities were eluted with 80% hexane-20% benzene. To elute the $\Delta^{5,7,24}$ compound, 60% hexane-40% benzene was used; it was followed by a small amount of more strongly adsorbed material, possibly the $\Delta^{25}$ analog, arising from an impurity in the starting material desmosterol. After saponification and column chromatography on silicic acid-Super Cel, the $\Delta^{5,7,24}$-$[3\alpha^3H]$-cholestatrien-3β-ol had a melting point of 106.5–108°, ultraviolet spectrum, at 272 nm, $\epsilon$: 10,300, at 282 nm, $\epsilon$: 11,500, at 283 nm, $\epsilon$: 6,350. The infrared spectrum is compatible with that reported by Scallen (8). The band at 881 cm$^{-1}$, indicative of a terminal double bond, is absent in this preparation.

The 5,6 double bond in $\Delta^{5,7,24}$-$[3\alpha^3H]$-cholestatrien-3β-ol was selectively hydrogenated using tris(triphenylphosphine)rhodium chloride as catalyst (9). Ten milligrams of sterol were dissolved in 10 ml of benzene, 50 mg of rhodium catalyst were added, and the hydrogenation was conducted at room temperature and atmospheric pressure for 3.5 hours. Under these conditions 10 to 15% of the 24,25 double bond was also hydrogenated,
while the reaction of the 5,6 double bond was about 90% complete. The catalyst was removed by passing the solution through a silicic acid-Super Cel column, 1 x 10 cm, and eluting the sterols with an additional 50 ml of benzene. The sterol mixture was separated on a silver nitrate column (5) using benzene as eluent. Fractions of 5 ml were collected. Fractions 9 to 12 contained material with the same chromatographic mobility as Δ7-cholesten-3β-ol. Fractions 14 to 20 were collected and the sterol crystallized from 1.5 ml of methanol at −20°C. The yield was 5 mg, m.p. 108–109°C. The sterol has no specific absorption in the 220–300-nm region. The molecular ion mass measured with a high resolution mass spectrometer was 384.3355 compared to the calculated value of 384.3394 for C27H46O.

Infrared Spectrometry—The infrared spectrum of synthetic Δ7,24-[3α-3H]cholestadien-3β-ol is compared with the spectrum of Δ7-cholesten-3β-ol in Fig. 1. In the 1300 to 1400 cm⁻¹ region, assigned to methyl and methylene bending vibrations, the spectrum of the Δ7,24-sterol, compared to the saturated side chain analog, shows the characteristic decrease in the intensity of the 1368 cm⁻¹ band compared to its higher frequency companion at 1378 cm⁻¹ (10). In the 800 to 900 cm⁻¹ region, assigned to the out-of-plane bending vibrations of hydrogen atoms attached to doubly bonded carbon, an increase in the intensity of the 825 cm⁻¹ band compared to the 843 cm⁻¹ band is noticeable with the introduction of the 24 double bond. This may be related to a low intensity band observed in Δ7-cholesten-3β-ol at 824 cm⁻¹ (10). The infrared spectrum of the synthetic Δ7,24-sterol is compatible with that of biological sterol (3).

Mass Spectrometry—The mass spectra of Δ7 and Δ7-cholesten 3β- and Δ7,24- and Δ7,24-[3α-3H]cholestadien-3β-ol are shown in Fig. 2. According to Wyllie and Dierassi (11), a diagnostically important cleavage is associated with the presence of the 24 double bond in the sterol side chain. The intense peak at m/e 271, corresponding to the loss of the side chain together with 2 hydrogen atoms from the steroid nucleus, appears in the Δ7,24- and Δ7,24-sterols, but is absent in the corresponding saturated side chain sterols. The triplet of peaks at m/e 299, 300, and 301, also assumed characteristic of the Δ7 unsaturation, is present in the Δ7,24-sterol, although of lower intensity than in the Δ7,24-compound.

It has been shown in several instances that the position of the nuclear double bond has some influence on the fragmentation pattern induced by the side chain double bond. The m/e 271 ion, or its equivalent at m/e 343 in the trimethylsilyl ethers, is present in both Δ7 and Δ7 analogs of the 24 methylene and 24-ethylidine sterols, the m/e 343 peak being the base peak in the Δ7 compounds (12). The double bond in position 8,9, however, has a definite inhibiting effect on the double hydrogen transfer in the 17-20 side chain fragmentation of zymosterol (13).

The base peak at m/e 69 of Δ7,24-cholestadien-3β-ol corresponds to the allylic cleavage of the 22-23 bond (14). In sterol acetates this base peak is characteristic for 24 unsaturation. The saturated side chain sterols have their base peak at m/e 43. In trimethylsilyl derivatives the m/e 69 ion is of no diagnostic value (13). In the free sterols, Fig. 2, the m/e 69 fragment is of considerably higher relative intensity in the Δ7,24-sterol than in its Δ7 analog, and may be characteristic of the Δ7,24 configuration. More information about the fragmentation patterns of free sterols is needed before any definite conclusions can be drawn.

Biological Studies—The biological conversion of Δ7,24-[3α-3H]-cholestadien-3β-ol to cholesterol and some intermediates was carried out in a Bucher homogenate (15) of rat liver. The incubation beakers contained 2 ml of cell-free homogenate in 0.1 M phosphate buffer, pH 7.3, 2 mg of TPNH, and 30 μg of Δ7,24-[3α-3H]cholestadien-3β-ol, specific activity 1200 cpm per μg, in 40 μl of dioxane. The incubation was carried out at 37°C under oxygen for 20 min in a Dubroff shaker. The incubation mixture was saponified to insure the absence of any sterol esters formed during the incubation. The free sterols were acetylated and analyzed by chromatography on a silver nitrate-impregnated alumina column (6, 7) together with 2 mg each of marker sterols Δ7- and Δ7-cholesten-3β, Δ7,24- and Δ7,24-cholestadien-3β- and Δ5, 7, 24-cholestatrien-3β-ol.1 The Δ7 and Δ7-sterol acetates were eluted with 90% hexane-10% benzene, Δ7,24- and Δ5, 7, 24- acetates with 80% hexane-20% benzene, the Δ7-acetate with 60% hexane-40% benzene, and the Δ5, 7, 24-acetate with 20% hexane-80% benzene. The chromatographic separation of the sterols is shown in Fig. 3, the composition of the sterol mixture is given in Table I. The relatively small amounts of Δ7- and Δ7- intermediates compared to the Δ7,24- and Δ5, 24-compounds indicate the preference of the unsaturated side chain pathway in cholesterol synthesis under the conditions used.

1 Isolated according to the method of Scallen (16) from the lungs of a pig treated with AY9044 and SC-12937.

| TABLE 1 Composition of sterol mixture obtained by enzymatic conversion of Δ7,24-[3α-3H]cholestadien-3β-ol in cell-free preparation of rat liver | Sterol | Recovered cpm% |
|---|---|---|
| Δ7,24 | 18.1 | % |
| Δ7,24 | 17.5 | 26.8 |
| Δ7 | 31.2 | 3.5 |
| Δ5,7 | 3.0 | 95% |

* Chromatographic recovery, 95%.
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