ISOLATION OF $\Delta^{8(14)}$-CHOLESTEN-3β-OL FROM RAT SKIN[1]

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In recent studies of the mechanism of the enzymatic removal of the methyl group attached to carbon atom 14 of cholesterol precursors two groups have reported that $\Delta^{8(14)}$-sterols serve as efficient precursors 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 to cholesterol has also been demonstrated. In this communication, we wish to present evidence indicating that $\Delta^{8(14)}$-cholesten-3β-ol is present in rat skin. This report constitutes the first isolation of this compound from animal tissues.

Experimental Procedure and Results

The general procedures and the preparation of the reference sterols used in this work have been described previously (Paliokas and Schroepfer, 1968; Lee et al., 1969).

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The skins of female Sprague-Dawley rats were extracted with acetone by a modification of the procedure of Frantz et al. (1957). The digitonin-precipitable sterols were isolated from the nonsaponifiable material of the skin extract and a portion (88 mg) of this material was acetylated with [^{3}H]-acetic anhydride (22.5 mg; 3,570 mc per mmole which was diluted with 200 µl of unlabeled acetic anhydride) in dry pyridine. Water was added and the steryl acetates (98 mg; 1.61 x 10^7 cpm per mg) were recovered by extraction with petroleum ether. A portion of the acetylated

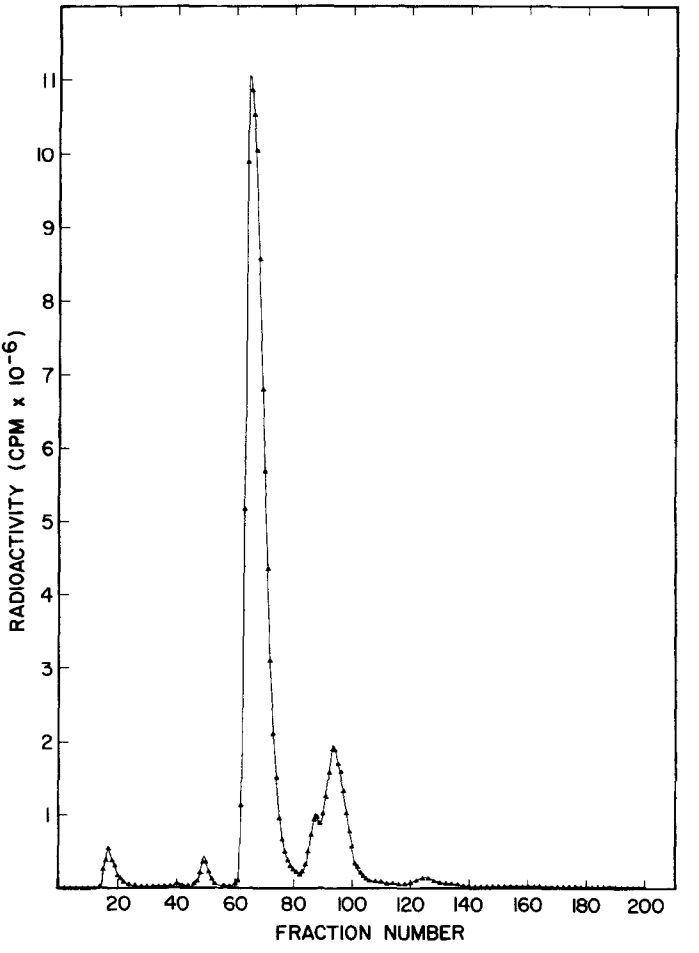

Figure 1. *Chromatography of* [^{3}H]*-acetate derivatives of digitonin-precipitable sterols of rat skin on a silicic acid-Super Cel column.*

sterol mixture (20 mg) was applied to a column of silicic acid-Super Cel (2:1; 100 x 1 cm). Using a mixture (16:84) of benzene and petroleum ether as the eluting solvent, fractions 4.2 ml in volume were collected. The resulting chromatogram is shown in Figure 1. The major peak of radioactivity (center at fraction 65) corresponds in mobility to that of cholesteryl acetate. The contents of fractions 70-200 were pooled (eliminating steryl acetates which have one or more additional methyl groups (C_{28}, C_{29}, and C_{30} sterols) which are eluted prior to cholesteryl acetate in this type of chromatography) and applied to a column of neutral alumina-Super Cel-silver nitrate (100 x 1 cm; Lee et al., 1969) along with 3β-acetoxy-$\Delta^{8(14)}$-cholestene (2.8 mg) and 3β-acetoxy-Δ^{7}-cholestene (2.7 mg). Using a mixture of n-hexane and benzene (90:10) as the eluting solvent, fractions 3.6 ml in volume were collected. The contents of fractions 40-60, corresponding to the mobility of the carrier 3β-acetoxy-$\Delta^{8(14)}$-cholestene, were pooled and applied to a second column of the same dimensions. Again the material corresponding to the mobility of the carrier 3β-acetoxy-$\Delta^{8(14)}$-cholestene was pooled and applied to a third column of neutral alumina-Super Cel-silver nitrate of the same dimensions along with 3β-acetoxy-Δ^{7}-cholestene (2.2 mg), 3β-[1-^{14}C]-acetoxy-cholestane (4 μg; 12,000 cpm), and 3β-[1-^{14}C]-acetoxy-Δ^{8}-cholestene (13.5 μg; 15,000 cpm). Fractions 3.0 ml in volume were collected. The resulting chromatogram is shown in Figure 2. The elution of the tritium-labeled material coincides closely with the profile of authentic 3β-acetoxy-$\Delta^{8(14)}$-cholestene, but the peak due to tritium is clearly wider than that expected for a single compound in this region of the chromatogram. The contents of fractions 41-46 were pooled and diluted with authentic 3β-acetoxy-$\Delta^{8(14)}$-cholestene. After two recrystallizations from methanol the specific activities of the crystals and the mother liquor were the same (approximately 50% of the initial specific activity). Conversion of this material to 3β-acetoxy-cholestane via 3β-acetoxy-Δ^{14}-cholestene

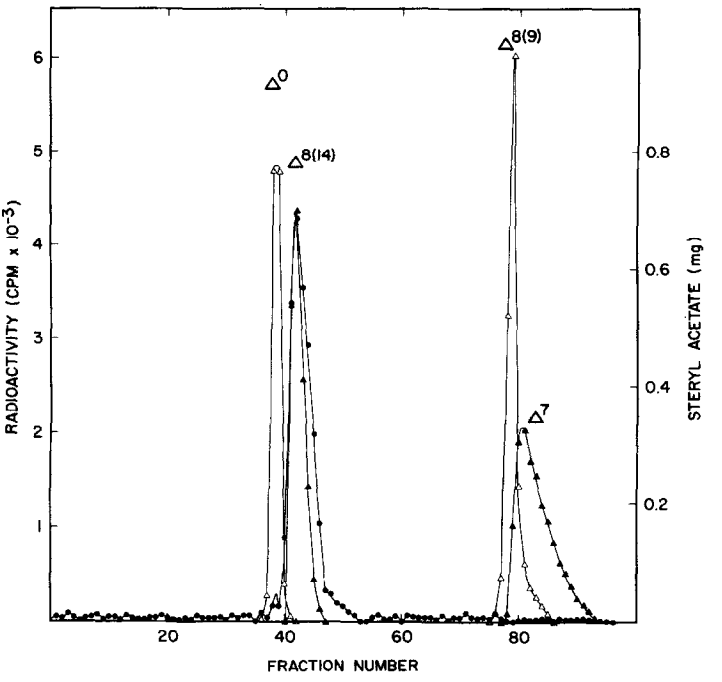

Figure 2. *Chromatogram illustrating similar mobility of the [^3H]-acetate of the isolated compound and 3β-acetoxy-$\Delta^{8(14)}$-cholestene on an alumina-Super Cel-silver nitrate column.*

●——●, *^3H radioactivity.*

Δ——Δ, *^{14}C radioactivity. The first ^{14}C peak is due to 3β-[1-^{14}C]-acetoxy-cholestane and the second is due to 3-[1-^{14}C]-acetoxy-Δ^8-cholestene.*

▲——▲, *steryl acetate determined colorimetrically. The first peak is due to 3β-acetoxy-$\Delta^{8(14)}$-cholestene and the second is due to 3β-acetoxy-Δ^7-cholestene.*

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employing published methods (Cornforth et al., 1957; Schenck et al., 1936; Mosbach et al., 1963) occurred without change in specific activity.

Another portion of the crude [^{3}H]-acetylated sterols was subjected to chromatography on a silicic acid-Super Cel column and repeated chromatography on alumina-Super Cel-silver nitrate columns as described above. The tritiated material corresponding to the mobility of 3β-acetoxy-$\Delta^{8(14)}$-cholestene was subjected to gas-liquid radiochromatographic

analysis on a column of 3% QF-1 on Gas-Chrom Q. Approximately 50% of the radioactivity due to steryl acetates was associated chromatographically with 3β-acetoxy-$\Delta^{8(14)}$-cholestene. Dilution of the tritiated material corresponding to the 3β-acetoxy-$\Delta^{8(14)}$-cholestene (after gas-liquid chromatography) with authentic carrier and repeated recrystallization from methanol and acetone-water resulted in no change in specific activity.

Discussion

Analysis of rat skin, a source of significant quantities of sterol precursors of cholesterol (Clayton et al., 1963), revealed the presence of a digitonin-precipitable sterol which showed a number of properties expected for $\Delta^{8(14)}$-cholesten-3β-ol.

The compound, in the form of the [^{3}H]-acetate derivative:

(1) cocrystallized with authentic 3β-acetoxy-$\Delta^{8(14)}$-cholestene from methanol and acetone-water,

(2) co-chromatographed with authentic 3β-acetoxy-$\Delta^{8(14)}$-cholestene on columns of alumina-Super Cel-silver nitrate,

(3) co-chromatographed with the authentic reference upon gas-liquid chromatographic analysis on a QF-1 column,

(4) was convertible to 3β-acetoxy-cholestane upon appropriate chemical treatment.

Previous studies of the sterols of rat skin failed to detect the presence of $\Delta^{8(14)}$-cholesten-3β-ol. The methods available for these studies did not allow sufficient resolution of Δ^{8}-cholesten-3β-ol and $\Delta^{8(14)}$-cholesten-3β-ol to permit the detection of the $\Delta^{8(14)}$-compound in the amount present (approximately 5-10% of the amount of Δ^{8}-cholesten-3β-ol) in rat skin.

Zalkow et al. (1968) have recently reported the isolation of a $\Delta^{8(14)}$-sterol, $\Delta^{8(14),22}$-stigmastadien-3β-ol, from the "rayless goldenrod" (Aplopappus heterophyllus).

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