INHIBITION OF HEPATIC STEROL SYNTHESIS AND REDUCTION OF SERUM CHOLESTEROL IN RATS BY 5α-CHOLEST-8(14)-EN-3β-OL-15-ONE*.

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Summary: The preparation of 5α-cholest-8(14)-en-3β-ol-15-one from 3β-benzoyloxy-5α-cholest-8(14)-en-15-one is described herein. Subcutaneous administration of the former compound (2 mg per day for 15 days) resulted in a significant depression of the incorporation of the label of [2-^{14}C]-acetate, but not of [2-^{14}C]-3RS-mevalonate, into digitonin-precipitable sterols in rat liver homogenate preparations. Subcutaneous administration of the inhibitor, 2 mg per day or 5 mg per day, for 3 days resulted in a 12% and 22% reduction of serum cholesterol levels, respectively.

The results of a recent study (1) indicate that a number of 15-oxygenated sterols are very potent inhibitors of sterol synthesis as judged by the incorporation of the label of [1-^{14}C]-acetate into digitonin-precipitable sterols and reduction of the activity of HMG-CoA reductase[1] in L-cells and in primary cultures of liver cells. 5α-Cholest-8(14)-en-3β-ol-15-one effected a 50% inhibition of sterol synthesis in L-cells at 10^{-7} M and a 50% reduction of the activity of HMG-CoA reductase at 3×10^{-7} M (1). The purposes of this communication are to describe the preparation of 5α-cholest-8(14)-en-3β-ol-15-one from 3β-benzoyloxy-5α-cholest-8(14)-en-15-one and to describe the effects of subcutaneous injection of the former compound in intact rats on hepatic sterol synthesis and serum cholesterol levels.

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1. β-Hydroxy-β-methyl glutaryl coenzyme A reductase.

Materials and Methods

Male rats of the Sprague-Dawley strain (110-190 g in weight) were used in this study and fed Purina rat chow ad libidum. The rats were maintained on a light (7 A.M. - 6 P.M.) -- dark (6 P.M. - 7 A.M.) cycle. Colorimetric assay of cholesterol was performed according to the method of Abell et al. (2)[2]. Protein was measured according to Goa (3). 5α-Cholest-8(14)-en-3β-ol-15-one (2.0 mg in 0.2 ml olive oil or 5.0 mg in 0.5 ml of olive oil) was administered subcutaneously once a day at ∿12 P.M. Control animals received injections of olive oil. Homogenates of rat liver were prepared as described previously (4) except that nicotinamide (30 mM) and $MgCl_2$ (5 mM) were included in the homogenization medium. The 10,000 x g supernatant fraction of the liver homogenate was isolated as described previously (5) and incubated for 2 hours at 37° in an oxygen atmosphere with either [2-^{14}C]-acetate (12 μCi; 3.05 mM; specific activity, 2 mCi/mmole) or [2-^{14}C]-3RS-mevalonate (1.7 μCi; 0.103 mM; specific activity, 8.22 mCi/mmole) in the presence of NAD (1 mM), NADP (1 mM), glucose-6-phosphate (3 mM), and ATP (5 mM). The unsaponifiable material was isolated as described previously (5) and, after the addition of carrier cholesterol, the digitonin-precipitable material was isolated (6). The free sterols were reisolated from the digitonide as described elsewhere (7). Aliquots were taken for assay of radioactivity and cholesterol before and after this purification step.

Blood samples (tail vein) were taken between 10 and 11 A.M. after 3 days of treatment. Rats were killed between 8 and 9 A.M. on the day following the last injection. 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one was prepared as described previously (8).

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2. Under the conditions of the colorimetric assay for cholesterol, 5α-cholest-8(14)-en-3β-ol-15-one gives a color response (optical density per mg of sterol at 620 nm 30 minutes after the addition of the reagent) of ∿0.02, a value approximately 1.3% that given by cholesterol.

Conversion of 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one to 5α-Cholest-8(14)-en-3β-ol-15-one

3β-Benzoyloxy-5α-cholest-8(14)-en-15-one (1.0 g) was dissolved ethanol (350 ml) and water (20 ml) and concentrated sulfuric acid (60 ml) were successively added and the resulting mixture was heated under reflux for 12 hours, cooled, reduced to 1/2 of its volume under reduced pressure, and diluted with 0.5 M NaCl (1,000 ml). The resulting precipitate was collected and recrystallized twice from acetone-methanol-water. The crystals were dissolved in acetone and warmed in the presence of Norite A for 15 min. The solution was filtered through Hyflo Super-Cel (Johns-Manville Corp.) and the Super-Cel was washed with methanol (twice the volume of acetone). The acetone and methanol solutions were combined and, after the addition of water, the crystalline product which formed was collected and dried in vacuo to give 5α-cholest-8(14)-en-3β-ol-15-one (660 mg; 83% yield) which melted at 147.5-149.0°; i.r.[3], ν_{max} (KBr) 3350, 1704, 1620 cm^{-1}; u.v.[4], λ_{max} (ethanol) 258 nm (ε=13,600); n.m.r.[5] (δ, p.p.m.) 1.2 (m, methylene envelope), 3.66 (m, 1H, C-3-H), 4.18 (m, 1H, C-7-H); m.s.[6], 400 (M; 29%), 385 (M-CH_3; 9%), 382 (M-H_2O; 5%), 367 (M-CH_3-H_2O; 18%), 287 (M-side chain; 17%), 269 (M-side chain-H_2O; 61%), 55 (100%); high resolution m.s., 400.3554 (calc. for $C_{27}H_{44}O_2$: 400.3541). The compound showed a single component on t.l.c.[7] analyses on silica gel G plates (solvent systems, 10% ether in benzene and 35% ethyl acetate in chloroform).

Effect of Subcutaneous Injection of 5α-Cholest-8(14)-en-3β-ol-15-one on Hepatic Sterol Synthesis and Serum Cholesterol Levels

After 15 days of treatment (2 mg per day in 0.2 ml of olive oil) significant reduction of the incorporation of the label of [2-^{14}C]-acetate, but

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3. i.r., infrared spectral analysis.
4. u.v., ultraviolet spectral analysis.
5. n.m.r., nuclear magnetic resonance spectral analysis.
6. m.s., mass spectra analysis.
7. t.l.c., thin-layer chromatography.

Table I. Effect of Subcutaneous Injection 5α-Cholest-8(14)-en-3β-ol-15-one on Synthesis of Digitonin-precipitable Sterols from:

Treatment	[2-^{14}C]-Acetate (dpm per mg. liver protein)	[2-^{14}C]-3RS-Mevalonate (dpm per mg. liver protein)
Control (N=6)	729 ± 110*	6,960 ± 1,200
Experimental (N=6)	227 ± 31 $p < 0.01$	7,460 ± 850 $p > 0.05$

*Standard error of mean

not of [2-^{14}C]-3RS-mevalonate, into digitonin precipitable sterols in rat liver homogenate preparations was observed (Table I). These findings are compatible with the results obtained in cell culture systems in which significant reduction of HMG-CoA reductase activity by the compound was observed.

After 3 days of subcutaneous injection of 5α-cholest-8(14)-en-3β-ol-15-one, significant reduction of serum cholesterol levels was observed (Table II). Analysis of the serum sterols by gas-liquid chromatography (3% OV-1 on Gas-Chrom Q) indicated no detectable (<1%) accumulation of sterols (including the administered 15-ketosterol) other than cholesterol.

There was no significant difference ($p > 0.30$) in growth of the rats treated with 2 mg per day of 5α-cholest-8(14)-en-3β-ol-15-one over a 15 day period. The average percentage weight gain in the experimental group (N=12) was 155% ± 3% (S.E.M.) while that in the control group (N=12) was 161% ± 5.2% (S.E.M.).

DISCUSSION

The chemical preparation of 5α-cholest-8(14)-en-3β-ol-15-one is described above. This compound has been shown to be a potent inhibitor of sterol biosynthesis in L-cells (1). The site of action of this inhibition appears in

Table II. Effect of Subcutaneous Injection of 5α-Cholest-8(14)-en-3β-ol-15-one on Serum Cholesterol Levels

Treatment	Serum Cholesterol (mg %)	Reduction (%)
Control (0.2 ml olive oil; N=12)	73.7 ± 2.9*	
Experimental (2.0 mg in 0.2 ml olive oil; N=12)	64.5 ± 2.5	12% ($p < 0.01$)
Control (0.5 ml olive oil; N=6)	79.5 ± 5.8	
Experimental (5.0 mg in 0.5 ml olive oil; N=6)	59.6 ± 2.2	22% ($p < 0.001$)

*Standard error of mean

L-cells appears to be at the level of HMG-CoA reductase (1). The present report describes the effects of subcutaneous injection (in olive oil) of 5α-cholest-8(14)-en-3β-ol-15-one on serum cholesterol levels and on hepatic sterol synthesis. Significant reduction of the incorporation of the label of [2-^{14}C]-acetate, but not of [2-^{14}C]-mevalonate, into digitonin-precipitable sterols by homogenate preparations of liver from rats treated with the 15-ketosterol was observed. Subcutaneous administration of the inhibitor, 2 mg per day or 5 mg per day, for 3 days resulted in a 12% and 22% reduction of serum cholesterol levels, respectively.

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