INHIBITION OF STEROL SYNTHESIS IN L CELLS BY 14α-ETHYL-5α-CHOLEST-7-EN-15α-OL-3-ONE AT NANOMOLAR CONCENTRATIONS*

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Summary: 14α-Ethyl-5α-cholest-7-en-15α-ol-3-one was prepared in 85% yield by selective oxidation of the 3β-hydroxyl function of 14α-ethyl-5α-cholest-7-en-3β,15α-diol by cholesterol oxidase. 14α-Ethyl-5α-cholest-7-en-15α-ol-3-one caused a 50% inhibition of the incorporation of [1-^{14}C]-acetate into digitonin-precipitable sterols at a concentration of 6 x 10^{-9} M in L cells and a 50% reduction in level of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase activity in the same cells at a concentration of 4 x 10^{-8} M.

The results of recent studies from these laboratories have indicated that a number of 15-oxygenated sterols are very potent inhibitors of sterol synthesis as judged by their effects on the incorporation of the label of [1-^{14}C]-acetate into digitonin-precipitable sterols and on the levels of HMG-CoA reductase activity in L cells and in primary cultures of fetal mouse liver cells (1-3). Moreover, one of these 15-oxygenated sterols, 5α-cholest-8(14)-en-3β-ol-15-one, has been found to result in reduction of serum cholesterol levels in rats upon subcutaneous administration (4) and to show profound hypocholesterolemic action upon dietary administration to rats and mice (5).

The purposes of this communication are to describe the preparation of 14α-ethyl-5α-cholest-7-en-15α-ol-3-one from 14α-ethyl-5α-cholest-7-en-3β,15α-diol and to report the results of studies of the effects of the former compound on sterol synthesis in L cells.

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Materials and Methods

Nuclear magnetic resonance spectra were recorded on a Perkin-Elmer HR-12 spectrometer at 60 MHz using $CDCl_3$ as solvent. Resonances are reported in p.p.m. (δ)downfield from the tetramethylsilane internal standard. Infrared spectra were recorded on a Beckman IR-9 spectrometer using KBr pellets. Low resolution mass spectra were recorded on a LKB-Model 9000S spectrometer. High resolution mass spectral analyses were recorded on a Varian CH-5 spectrometer through the courtesy of Professor C. C. Sweeley. Gas-liquid chromatographic analyses were made using a Hewlett-Packard Model 402 instrument equipped with dual flame ionization detectors. The column temperature was 270° and the carrier gas was helium (flow rate, 66 ml/min). Cholesterol oxidase (components 1 and 3 of the Cholesterol Auto Test) was purchased from Biodynamics (BMC Division) Boehringer Mannheim (Indianapolis, Indiana). 14α-Ethyl-5α-Cholest-7-en-3β,15α-diol and 14α-ethyl-5α-cholest-7-en-3β-ol-15-one were samples whose preparation was recently described elsewhere (3).

14α-Ethyl-5α-cholest-7-en-15α-ol-3-one

To component 1 (250 ml) of the Bio-Dynamics (BMC Division) Cholesterol Auto Test was added cholesterol oxidase (16 ml; component 3 of the Cholesterol Auto Test) and the resulting mixture was diluted with distilled water (734 ml). 14α-Ethyl-5α-cholest-7-en-3β,15α-diol (100 mg) in isopropanol was added and the resulting mixture was incubated with shaking at 37° for 4.5 hours. The mixture was extracted twice with chloroform (100 ml portions) and the volume of the combined extracts was reduced to ∿6 ml under reduced pressure. This material was subjected to preparative thin layer chromatography on silica gel G plates (750 microns in thickness) using ether as the developing solvent and the desired ketone was eluted from the plates with ether. The residue obtained upon evaporation of the solvent under reduced pressure was recrystallized twice from methanol-ether to give 14α-ethyl-5α-cholest-7-en-15α-ol-3-one (85.3 mg; 85% yield) melting at 114-115°; infrared, ν_{max} 3350, 2930, 1715 (C=O stretch in six-membered ring)[1], 1645, 1375,

[1]The corresponding carbonyl absorbance in the infrared spectrum of 14α-ethyl-5α-cholest-7-en-3β-ol-15-one occurs at 1736 cm^{-1} (3).

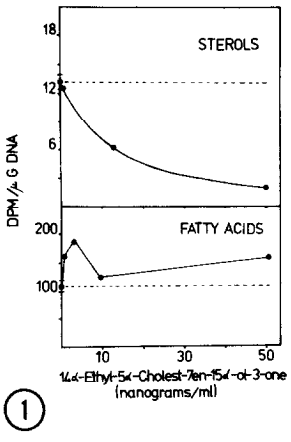
1260, 1120, and 1050 (C-O stretch) cm^{-1}; nuclear magnetic resonance, 4.35 (m, 1H, C-15-H), and 5.56 (m, 1H, C-7-H); low resolution mass spectrum, 428 (3%; M), 410 (3%; M-H_2O), 399 (25%; M-CH_2CH_3), 381 (100%; M-CH_2CH_3-H_2O), 297 (6%), 270 (7%), 258 (3%), 256 (5%), 244 (5%), 232 (5%), and 204 (19%); high resolution mass spectral analysis, 428.3667 (calculated for $C_{29}H_{48}O_2$: 428.3654). The compound showed a single component on gas-liquid chromatographic analysis on a 3% OV-17 column with a relative (to 5α-cholestane) retention time of 7.50. The compound was easily distinguished by gas-liquid chromatography from 14α-ethyl-5α-cholest-7-en-3β-ol-15-one and 14α-ethyl-5α-cholest-7-en-3β,15α-diol which showed relative retention times of 6.15 and 5.72, respectively. The 3-ketone showed a single component on thin layer chromatographic analyses on silica gel G plates (250 microns in thickness) in four different solvent systems: (ether; acetone; chloroform-ethyl acetate, 1:1; and chloroform-ethyl acetate, 65:45). In the latter solvent system, the R_f values of 14α-ethyl-5α-cholest-7-en-15α-ol-3-one, 14α-ethyl-5α-cholest-7-en-3β-ol-15-one, and 14α-ethyl-5α-cholest-7-en-3β,15α-diol were 0.52, 0.59, and 0.68, respectively.

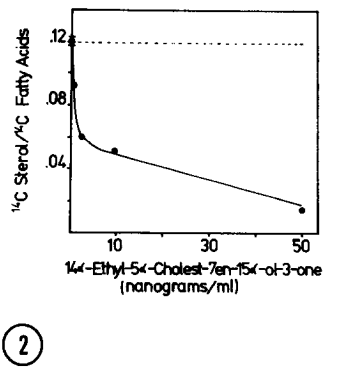
Cell Culture Studies

Mouse L cell (a subline of NCTC clone 929 mouse fibroblasts) cultures were grown in serum-free media as described previously (2,6,7). The preparation of steroid-containing media, procedures for assay of the rate of conversion of [1-^{14}C]-acetate into digitonin-precipitable sterols and fatty acids and methods for measurement of DNA, protein, and HMG-CoA reductase were as described previously (2,6,7). The L cell cultures were preincubated with the test compound for 4 hours; then [1-^{14}C]-acetate was added at a concentration of 4 μmoles (4 μCi) per ml. To determine the effects of the sterol on HMG-CoA reductase of the L cells, the sterol was incubated with the cultures for 5 hours prior to harvesting them for the determination of microsomal HMG-CoA reductase activity.

Results

The effects of 14α-ethyl-5α-cholest-7-en-15α-ol-3-one on the rates of incorporation of [1-^{14}C]-acetate into digitonin-precipitable sterols and fatty acids are





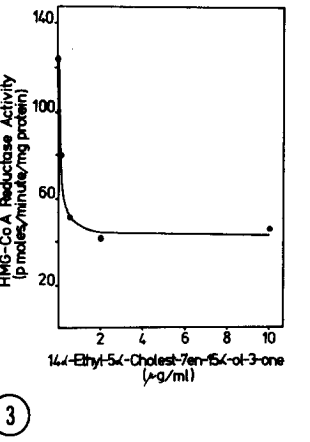


Figure 1. Effect of 14α-ethyl-5α-cholest-7-en-15α-ol-3-one on the rates of incorporation of [1-^{14}C]-acetate into digitonin-precipitable sterols and fatty acids in L cells grown in serum-free media (2,6,7).

Figure 2. Effect of 14α-ethyl-5α-cholest-7-en-15α-ol-3-one on the ratio of the incorporation of [1-^{14}C]-acetate into [^{14}C]-digitonin-precipitable sterols to the incorporation of [1-^{14}C]-acetate into fatty acids in L cells grown in serum-free media (2,6,7).

Figure 3. Effect of 14α-ethyl-5α-cholest-7-en-15α-ol-3-one on the level of microsomal HMG-CoA reductase activity in L cells grown in serum-free media (2,6,7).

shown in Figure 1. Studies of the rates of acetate metabolism to fatty acids were made so as to detect any possible effects of the inhibitor of sterol synthesis on general metabolism. In an effort to correct for variations of individual cultures upon the estimation of the inhibitory potency, the concentration required to inhibit sterol synthesis by 50% was estimated from a plot (Figure 2) of the ratio of [^{14}C] sterols to [^{14}C] fatty acids as a function of the concentration of the inhibitor (2, 6). The results of this analysis indicated that 14α-ethyl-5α-cholest-7-en-15α-ol-3-one caused a 50% inhibition of sterol synthesis at a concentration of 6 x 10^{-9} M. A major site of this inhibition of sterol synthesis appears to be at the level of HMG-CoA reductase (Figure 3) since 14α-ethyl-5α-cholest-7-en-15α-ol-3-one caused a 50% reduction in the level of microsomal HMG-CoA reductase activity in the L cells at a concentration of 4 x 10^{-8} M.

Discussion

The results of studies from several laboratories have shown that a number of oxygenated derivatives of cholesterol are potent inhibitors of the synthesis of digitonin-precipitable sterols in animal cells in culture (6-12). Recently we have found that a number of 15-oxygenated sterols are very potent inhibitors of sterol synthesis in L cells and in primary cultures of mouse liver cells (1-3). Prior to this study the most potent inhibitor of sterol synthesis of this type reported was 14α-ethyl-5α-cholest-7-en-3β, 15α-diol which caused a 50% inhibition of sterol synthesis in L cells at a concentration of 5×10^{-8} M (3). In the present study 14α-ethyl-5α-cholest-7-en-15α-ol-3-one has been found to be significantly more potent, causing a 50% inhibition of sterol synthesis in L cells at a concentration of 6×10^{-9} M. To our knowledge this oxygenated sterol is the most potent compound of this type in the inhibition of sterol synthesis in animal cells.

The utility of the enzyme cholesterol oxidase for the high yield selective oxidation of the 3β-hydroxyl function of 14α-ethyl-5α-cholest-7-en-3β, 15α-diol is also noteworthy.

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