INHIBITORS OF STEROL SYNTHESIS. SYNTHESIS OF [2,4-^{3}H]5α-CHOLEST-8(14)-ENE-3β,7α,15α-TRIOL AND [2,4-^{3}H]5α-CHOLEST-8(14)-EN-3β-OL-15-ONE

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S U M M A R Y

Oxidation of 5α-cholest-8(14)-ene-3β,7α,15α-triol with silver carbonate-celite gave 5α-cholest-8(14)-ene-7α,15α-diol-3-one in 88% yield. Treatment of the latter compound with tritiated water under basic conditions gave a labeled product which was reduced with lithium tri-tert-butoxyaluminum hydride in tetrahydrofuran to give [2,4-^{3}H]5α-cholest-8(14)-ene-3β,7α,15α-triol which was obtained in high purity after medium pressure liquid chromatography in the form of its triacetate derivative. Treatment of the labeled triol with concentrated hydrochloric acid in 95% ethanol gave the desired [2,4-^{3}H]5α-cholest-8(14)-en-3β-ol-15-one.

Key Words: 5α-cholest-8(14)-en-3β-ol-15-one, 5α-cholest-8(14)-ene-3β,7α,15α-triol, radiosynthesis

I N T R O D U C T I O N

5α-Cholest-8(14)-en-3β-ol-15-one (I; Figure 1) has been shown to be a potent inhibitor of sterol synthesis in L cells and in primary cultures of fetal mouse liver cells and causes a reduction in the level of HMG-CoA reductase in the same cells (1,2). This 15-oxygenated sterol has also been shown to have significant hypocholesterolemic activity upon administration to intact animals (3-5). 5α-Cholest-8(14)-ene-3β,7α,15α-triol (II) has also been found to be an inhibitor of sterol synthesis in animal cells in culture (1,2) but is considerably less potent in this respect than I. The structure of II has recently been established by the results of detailed ^{13}C nuclear magnetic resonance studies (6). For studies of the comparative metabolism, absorption, and distribution of I and II we required these compounds labeled with ^{3}H or ^{14}C. While labeled I and II could be

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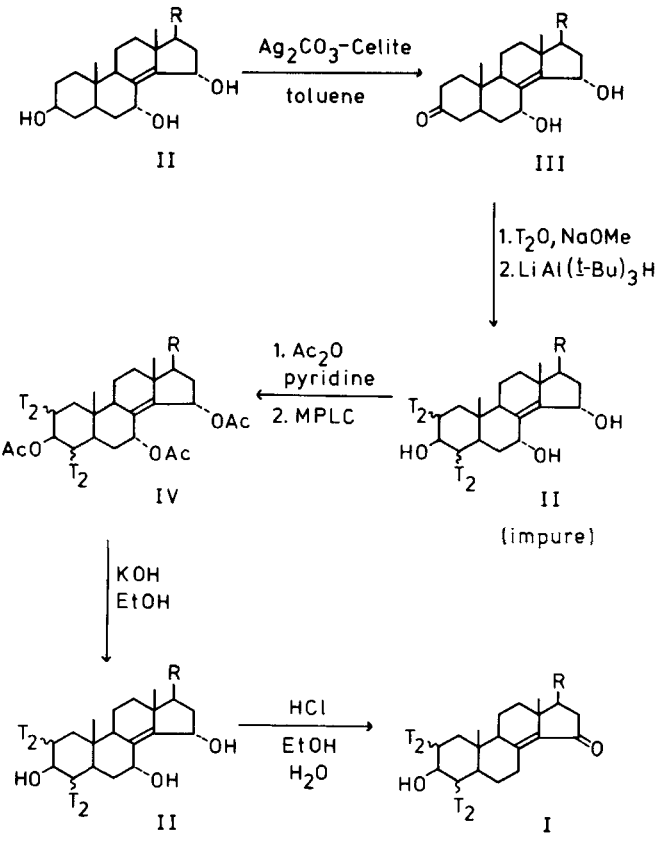

Figure 1. Synthesis of [2,4-^{3}H]5α-cholest-8(14)-ene-3β,7α,15α-triol (II) and [2,4-^{3}H]5α-cholest-8(14)-en-3β-ol-15-one (I).

prepared from commercially available labeled cholesterol, such an approach is less than ideal since a number of steps are required and it would be difficult to obtain labeled I and II of high specific activity. We have recently described (7) the preparation of [2,4-^{3}H]-I by the following approach: 5α-cholest-7,14-dien-3β-ol was converted to 5α-cholest-7,14-dien-3-one by oxidation with pyridinium chlorochromate; the $\Delta^{7,14}$-3-ketone was treated with m-chloroperbenzoic acid and the resulting crude product was treated with ethanolic KOH to give 5α-cholest-8(14)-ene-7α,15α-diol-3-one (III); III was labeled with ^{3}H by exchange with tritiated water under basic conditions; reduction of the latter compound with lithium aluminum hydride gave a mixture of [2,4-^{3}H]-II and its 3α-hydroxy epimer which were inseparable by chromatography; treatment of the mixture with hydrochloric acid in a mixture of $CHCl_3$ and methanol gave a complex mixture from which [2,4-^{3}H]-I was isolated by column chromatography. The purpose of this paper is to describe an alternative method which permits a more efficient and clean preparation of radiolabeled I and which also gives radiolabeled II in a high state of purity.

A key compound in the preparation of labeled I and II is the 7α,15α-hydroxy-$\Delta^{8(14)}$-3-ketone (III). In the present work we have prepared this compound by the single step, high yield oxidation of the 3β-hydroxyl function of II using a mixture of silver carbonate and celite (prepared according to Fetizon and Goldfier (8)). This approach to the preparation of III is clearly superior to the method utilized previously (7) and outlined above. We have previously utilized the silver carbonate-celite reagent for the selective oxidation of the 3β-hydroxyl function of several 3β,15-dihydroxysterols (9,10). After preparation of [2,4-^{3}H]-III by exchange with tritiated water under basic conditions, the 3-keto function was reduced with lithium tri-tert-butoxyaluminum hydride to give, almost exclusively, the 3β-hydroxy epimer ([2,4-^{3}H]-II), a result facilitated by the use of this metal hydride and the use of tetrahydrofuran as the solvent (11,12). The resulting labeled triol was found, by radio-GLC analysis of the tris-TMS derivative, to contain several contaminants which were not removed by conventional chromatog-

raphy. Purification was effected by conversion of the triol to its triacetate derivative which was purified by medium pressure liquid chromatography on a silica gel column. Saponification of the triacetate gave [2,4-^{3}H]-II of high purity. Treatment of [2,4-^{3}H]-II with hydrochloric acid gave, after chromatography and crystallization, [2,4-^{3}H]-I as the major product. The generation of a $\Delta^{8(14)}$-15-ketone system upon treatment of a $\Delta^{8(14)}$-7α,15α-dihydroxysterol or a $\Delta^{8(14)}$-3β,7α,15α-trihydroxysterol has been described previously (6,7,13-15). For example, we have reported that 5α-cholest-8(14)-ene-3β,7α,15α-triol (II), upon heating with hydrochloric acid in ethanol, gave I in high (87%) yield (6). In the preparation of [2,4-^{3}H]-I, the $\Delta^{8(14)}$-7α,15α-dihydroxy system of II is effectively used as a "protecting" function from which the $\Delta^{8(14)}$-15-ketone system can be generated with ease. This feature permits introduction of the isotopic hydrogen at a chemically and, for our purposes, biologically stable position in the steroid nucleus at a point in the synthesis which permits the attainment of high specific activity in the final product with a minimum of chemical steps. This is in contrast to an approach which utilizes commercially available isotopically labeled cholesterol as the starting material for the radiochemical synthesis of labeled I and II.

EXPERIMENTAL

General

Authentic samples of unlabeled I (2), II (6), III (6), and IV (6) were prepared previously. TLC was performed on plates of silica gel G. GLC was, unless stated otherwise, performed on columns of 3% OV-1 on Gas-Chrom Q as described previously (2). Radio-TLC and Radio-GLC analyses were made as described previously (16). Routine column chromatography employed silica gel (60-200 mesh). Medium pressure (100 p.s.i.) liquid chromatography (MPLC) employed columns (118 cm x 1.5 cm) of silica gel (0.32-0.063 mm). The fraction size for all column chromatographic runs was 20 ml. The following solvent systems were employed for chromatogra-

phy: SS-1, 50% ethyl acetate in toluene; SS-2, 10% ethyl acetate in $CHCl_3$; SS-3, 50% ether in toluene; SS-4, 50% ethyl acetate in hexane; SS-5, ethyl acetate; SS-6, 20% ether in toluene; SS-7, 10% ether in toluene; SS-8, 25% ethyl acetate in toluene; and SS-9, 25% ether in toluene. Unless stated otherwise, organic extracts were routinely washed with water, dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure. Silver carbonate-celite was prepared according to Fetizon and Goldfier (8).

5α-Cholest-8(14)-ene-7α,15α-triol-3-one (III)

To II (1.00 g) in toluene (220 ml) was added silver carbonate-celite (6.6 g). The resulting mixture was heated to reflux for 4 h. After filtering the mixture, the solvent was evaporated under reduced pressure. Analysis by TLC (SS-2) indicated only traces of the starting material and that the product was ~97% pure. The residue was subjected to MPLC using SS-2 as the eluting solvent. The contents of fractions 43-78 were pooled and recrystallized from hexane to give III (0.87 g; 88% yield) melting at 121.0-122.5° C (lit., 121.5-122.5° C (6)). The IR, NMR, and MS were identical to those of III prepared by an independent method (6). The product showed a single component on TLC in 3 solvent systems (SS-1, SS-3, SS-4) and on GLC analysis of the free sterol and the bis-TMS derivative (3% OV-1 and 3% OV-17) with the same chromatographic behavior as authentic III and its TMS derivative.

[2,4-^{3}H]5α-Cholest-8(14)-ene-3β,7α,15α-triol ([2,4-^{3}H]-II) (crude)

III (500 mg), sodium methoxide (500 mg), tritiated water (100 μl; 100 mCi), and dry dioxane (20 ml) were heated in a sealed tube at 40° C for 6 h and then allowed to stand at room temperature for an additional 12 h. The reaction mixture was poured into a saturated NH_4Cl solution (400 ml) and extracted 6 times with ether containing CH_2Cl_2 (10%) (200 ml portions). The crude product was dissolved in dry tetrahydrofuran (50 ml). Lithium tri-tert-butoxyaluminum hydride (1.5 g) was added and, after standing for several hours

at room temperature, ice was cautiously added to decompose the excess hydride. The mixture was poured into a saturated NH_4Cl solution (600 ml) and extracted 12 times with ether containing CH_2Cl_2 (10%) (200 ml portions). The crude product residue was subjected to silica gel column (70 cm x 1.5 cm) chromatography using SS-5 as the eluting solvent. The contents of fractions 26-39 showed a single (99.6%) labeled component on radio-TLC analyses in 2 solvent systems (SS-1, R_f 0.08; SS-5, R_f 0.24) with the same chromatographic behavior as authentic II. Radio-GLC analysis of the tris-TMS derivative of the crude product showed one major component (mass and ^3H) with the same behavior as that of the same derivative of authentic II but with a total of ~8% mass and labeled contaminants.

[2,4-^{3}H]-3β,7α,15α-Triacetoxy-5α-cholest-8(14)-ene ([2,4-^{3}H]-IV)

The impure labeled triol (440 mg) from above was dissolved in pyridine (50 ml), acetic anhydride (50 ml) was added, and the solution was allowed to stand at 35° C for 24 h. Ice and water were added and the mixture was thoroughly extracted with ether. The combined extracts were successively washed with water, cold 5% HCl, water, 5% $NaHCO_3$, and water, dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure. The residue (492 mg) showed one major component on TLC (SS-6; R_f 0.34) with the same mobility as that of authentic IV. Three minor components (R_f 0.11, 0.29, and 0.40) were also observed. Radio-TLC showed that most (~80%) of the radioactivity had the same chromatographic mobility as authentic IV. The labeled triaceate (IV) was purified by MPLC using SS-7 as the eluting solvent. The contents of fractions 14-28 were pooled and, after evaporation of the solvent, gave [2,4-^{3}H]-IV (specific activity, 20.4 mCi per mmol) as a colorless glass (384 mg). TLC (SS-6) showed a single component with the same mobility (R_f 0.34) as that of authentic IV. Radio-TLC (same system) indicated a single labeled component (~99.6%) with the same mobility as that of authentic IV.

[2,4-^{3}H]5α-Cholest-8(14)-ene-3β,7α,15α-triol ([2,4-^{3}H]-II)

To [2,4-^{3}H]-IV (300 mg) in ethanol (40 ml) was added 18N KOH (5 ml). After stirring for 24 h at 30° C, the solvent was removed under reduced pressure, water was added, and the mixture was thoroughly extracted with ether containing CH_2Cl_2 (10%). The crude product was recrystallized from ethyl acetate-hexane at -15° C to give [2,4-^{3}H]-II (247 mg; 93% yield; specific activity, 21.3 mCi per mmol) melting at 213-214° C (lit. for unlabeled sterol, 213-214° C) (2) and 212.5-214.0° C (6)). The product showed a single component on TLC (SS-1; R_f 0.24) with the same mobility as authentic II. Radio-TLC (same system) showed a single (99.8%) labeled component with the same mobility as that of authentic II. Radio-GLC analysis of the tris-TMS derivative showed a single (99.6%) component with the same mobility as that of the same derivative of authentic II.

[2,4-^{3}H]5α-Cholest-8(14)-en-3β-ol-15-one ([2,4-^{3}H]-I

To [2,4-^{3}H]-II (190 mg) in 95% ethanol (40 ml) was added concentrated hydrochloric acid (2 ml). The resulting mixture was heated under gentle reflux for 2 h. Radio-TLC (SS-8) of the crude reaction mixture indicated one major (~62%) labeled component with the same mobility as authentic I (R_f 0.26). The solvent was removed under reduced pressure and the residual moisture was removed by the addition of ethanol and evaporation to dryness under reduced pressure. The resulting residue was subjected to silica gel column (70 cm x 2 cm) chromatography using SS-8 as the eluting solvent. The contents of fractions 68-132 were pooled and recrystallized from methanol-water to give [2,4-^{3}H]-I (88.3 mg; 49% yield; specific activity, 19.9 mCi per mmol) melting at 147.5-149.0° C (lit. for unlabeled sterol, 147.5-149.0° C (3,6)). The product showed a single component on TLC in 2 solvent systems (SS-9; R_f 0.14; SS-8, R_f 0.26) with the same mobility as that of authentic I. Radio-TLC (same systems) showed a single (>99%) component with the same mobility as that of authentic I. Radio-GLC showed one (>97%) labeled component with the same mobility as that of authentic I.

REFERENCES

1. Schroepfer G. J., Jr. Parish E. J. Chen H. W. and Kandutsch A. A. - Fed. Proc. 35, 1697 (1976).
2. Schroepfer G. J., Jr. Parish E. J. Chen H. W. and Kandutsch A. A. - J. Biol. Chem. 252, 8975 (1977).
3. Raulston D. L. Mishaw C. O. Parish E. J. and Schroepfer G. J., Jr. - Biochem. Biophys. Res. Commun. 71, 984 (1976).
4. Schroepfer G. J., Jr. Monger D. J. Taylor A. S. Chamberlain J. S. Parish E. J. Kisic A. and Kandutsch A. A. - Biochem. Biophys. Res. Commun. 78, 1227 (1977).
5. Kisic A. Taylor A. S. Chamberlain J. S. Parish E. J. and Schroepfer G. J. Jr. - Fed. Proc. 37, 1663 (1978).
6. Tsuda M. Parish E. J. and Schroepfer G. J., Jr. - J. Org. Chem. 44, 1282 (1979).
7. Monger D. J. Parish E. J. and Schroepfer G. J., Jr. - J. Biol. Chem., in press.
8. Fetizon M. and Goldfier M. - Compt. Rend. 267, 900 (1968).
9. Schroepfer G. J., Jr. Parish E. J. and Kandutsch A. A. - Chem. Phys. Lipids 25, 265 (1979)
10. Parish E. J. and Schroepfer G. J., Jr. - Chem. Phys. Lipids 25, 381 (1979).
11. Wheeler O. H. and Mateos J. L. - Chem. Ind. (London) 395 (1957).
12. Haubenstock H. and Hong S.-J. - Tetrahedron 34, 2445 (1978).
13. Woodward R. B. Patchett A. A. Barton D. H. R. Ives D. A. J. and Kelly R. B. - J. Amer. Chem. Soc. 76, 2852 (1954).
14. Woodward R. B. Patchett A. A. Barton D. H. R. Ives D. A. J. and Kelly R. B. - J. Chem. Soc. 1131 (1957).
15. Muccino R. B. and Djerassi C. - J. Amer. Chem. Soc. 96, 556 (1974).
16. Paliokas A. M. and Schroepfer G. J., Jr. - J. Biol. Chem. 243, 453 (1968).