BILE ACIDS. LXXI.

A NEW SYNTHESIS OF

(25R)-3α,7α-DIHYDROXY-[5α,6α-$^3H_2$]-5α-CHOLESTAN-26-OIC ACID (1)

Daniel M. Tal (2) and William H. Elliott*

Edward A. Doisy Department of Biochemistry

Saint Louis University School of Medicine

St. Louis, MO 63104

SUMMARY

Tritiation of 7-oxo-5-cholestene-3β,26-diol diacetate afforded a mixture of [5α,6α-$^3H_2$] products which were reduced with lithium aluminum hydride to provide a mixture of [5α,6α-$^3H_2$]-3β,(7α and 7β), 26-triols and 3β, 26-diol. Oxidation with Jones reagent provided 3,7-dioxo and 3-oxo-[5α,6α-$^3H_2$]-5α-cholestanoic acids which were separated. Stereospecific reduction of the 3,7-dioxo methyl ester with K-Selectride followed by alkaline hydrolysis afforded (25R)-3α,7α-dihydroxy-[5α,6α-$^3H_2$]-5α-cholestan-26-oic acid.

Key Words: (25R)-3α,7α-dihydroxy-[5α,6α-$^3H_2$]-5α-cholestan-26-oic acid, 7-oxo-5-cholestene-3β,26-diol diacetate, K-Selectride, methyl 3,7-dioxo-[5α,6α-$^3H_2$]-5α-cholestanoate, dihydroxy [5α,6α-$^3H_2$]-5α-C_{27} bile acid.

INTRODUCTION

The biosynthesis of bile acids from sterols includes a number of chemical changes in the steroid nucleus prior to transformation of the C_{27} sterol into a C_{24} carboxylic acid (3). To ascertain whether a similar pathway occurs in formation of 5α- (or allo-) bile acids (4), a sample of labeled 5α-C_{27} acid was needed. Although a synthesis of (25R)-3α,7α-dihydroxy-[5α,6α-$^3H_2$]-5α-cholestan-26-oic acid has been reported (5), a new and improved synthesis of the above radioactive and non-radioactive acids has been developed.

* Author to whom correspondence is to be addressed.

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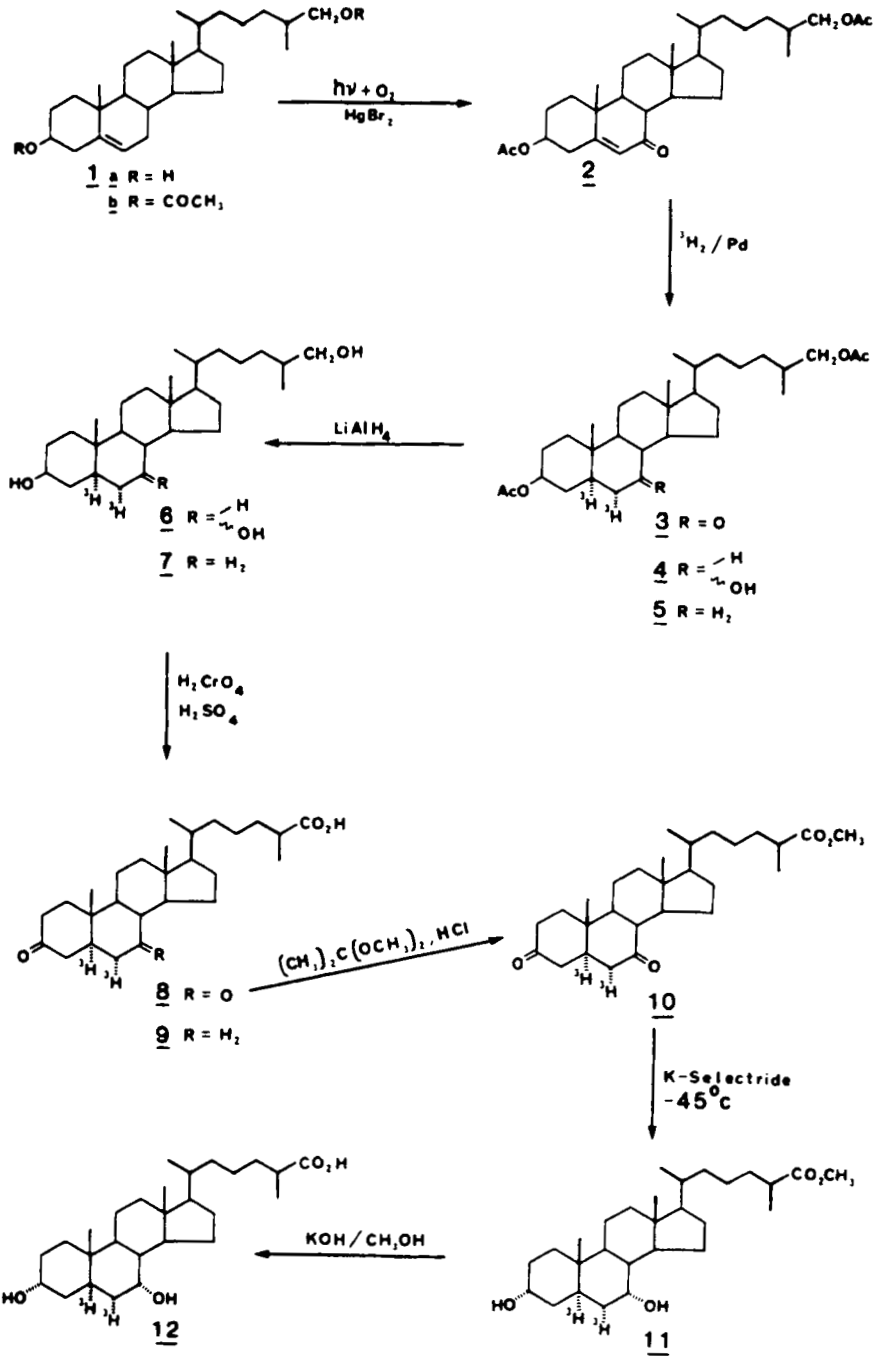
CH₂OR
RO
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b R = COCH₃
hν + O₂
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AcO
O
2
³H₂ / Pd
CH₂OAc
AcO
R
3 R = O
4 R = H, OH
5 R = H₂
LiAlH₄
CH₂OH
HO
R
6 R = H, OH
7 R = H₂
H₂CrO₄
H₂SO₄
CO₂H
O
R
8 R = O
9 R = H₂
(CH₃)₂C(OCH₃)₂, HCl
CO₂CH₃
O
O
10
K-Selectride
-45°C
CO₂CH₃
HO
OH
11
KOH / CH₃OH
CO₂H
HO
OH
12

DISCUSSION

Since the R-configuration at the C-25 chiral center of the desired 5α-C_{27} acid was preferred for biochemical studies, kryptogenin [(25R)-3β,26-dihydroxy-cholest-5-ene-16,22-dione] was chosen as the starting material for the synthesis since the chirality at C-25 has been established (6-8). In accord with the recommendations of Popják *et al.* (9) the location of the carboxyl group in the desired acid (12) was assigned as position 26. After esterification and separation of commercial kryptogenin diacetate from yamogenin and diosgenin acetates by preparative HPLC (10) the product was subjected to successive Clemmensen and Wolff-Kishner reductions (11,12) to provide (25R)-26-hydroxycholesterol (1a).

The diacetate (1b) was subjected to a photochemical allylic oxidation (13) to provide 7-oxo-26-hydroxycholesterol diacetate (2), which was used for the non-radioactive and radiochemical synthesis of the acid (12). The intermediate products derived from preparation of the non-radioactive analog of acid 12 were used as standards for comparison with those intermediates in the sequence with tritiated material.

After heterogeneous catalytic reduction of the enone 2 with 5 Ci of tritium gas over 5% palladium on carbon (5) (New England Nuclear Corp., Boston, MA), a product with a specific activity of 25.0 m Ci/mg was obtained. An aliquot containing (25R)-[5α,6α-$^3H_2$]-5α-cholestane-3β,26-diol-7-one diacetate (3, 61%), (25R)-[5α,6α-$^3H_2$]-3β,7(α and β),26-trihydroxy-5α-cholestane 3,26-diacetate (4, 36%) and (25R)-[5α,6α-$^3H_2$]-5α-cholestane-3β,26-diol diacetate (5, 3%), was diluted with 100 mg of a mixture of the non-radioactive materials and reduced with an excess of lithium aluminum hydride in ether to afford a mixture of (25R)-3β,7(α and β),26-trihydroxy-[5α,6α-$^3H_2$]-5α-cholestane (6) and (25R)-[5α,6α-$^3H_2$]-5α-cholestane-3β,26-diol (7). Tlc of a sample showed two major radioactive spots (isomers of 6,) and a minor spot at R_f 0.34 (compound 7), by comparison with non-radioactive standards.

The mixture of compounds (6 and 7) was further oxidized with H_2CrO_4 and H_2SO_4 in acetone (14) to provide two major products (25R)-[5α,6α-$^3H_2$]-5α-cholestane-3,7-dion-26-oic acid (8), (25R)-[5α,6α-$^3H_2$]-5α-cholestan-3-on-26-oic acid (9) and traces of less polar compounds. Since the same product (8) was

obtained from three of the precursors (3 and 4 with 7α- and 7β-OH), resolution of 8 and 9 was carried out at this stage by preparative layer chromatography (plc). The fraction containing the dioxo acid (8) retained 38% of the total tritium.

Some tritium was undoubtedly present at position 7 in compounds 4, 5, 6, 7 and 9, and to a minor extent at position 8 in all the tritiated compounds (15). However, the 7-deoxygenated products (5, 7 and 9) were present in very small quantities and in any case were later separated from the main product. The 7ξ-alcohols (4, and 6) were oxidized to 7-oxo products, thus eliminating the tritium at C-7.

Methylation of acid 8 with 2,2-dimethoxypropane (16) provided the ester 10. From tlc and radioassay, 74% of initial activity was found in the fraction with R_f identical (16) to that of the non-radioactive ester (10).

The simultaneous stereoselective reduction of the C-3 and C-7 oxo groups was achieved (17) with potassium tri-sec-butylborohydride (K-Selectride, Aldrich). After the usual work-up, identity of the products was verified by tlc; radio-assay showed that 69% of the tritiated material was present in compound 11 (in the non-radioactive preparation an 82% yield (GLC) of product was obtained). R_f's of the non-radioactive esters and the tritiated ester 11 were identical.

The methyl ester (11) was hydrolyzed with 5% methanolic potassium hydroxide to the free acid 12, with a specific activity of 0.146 mCi/mg or 63.5 mCi/mmol. The R_f's of radioactive and non-radioactive acids were identical; the properties of the non-radioactive acid were identical to those reported (5,8).

EXPERIMENTAL

Preparative HPLC was performed on a PrepLC/System 500 apparatus (Waters Assoc.) with silica columns. Semi-prep HPLC was carried out with a Waters 201 System (10) with a Waters μPorasil column (7.8mm x 30 cm). Tlc was done on silica gel 60 F-254 (0.2 mm thickness) with aluminum support (E. Merck, Germany); preparative layer chromatography (plc) was done on silica gel 60 F-254 (2mm thickness) on glass plates. Melting points were carried out on a Fisher-Johns apparatus and are uncorrected. Radioactivity was measured in a Tracor

Analytical 6892 liquid scintillation spectrometer. The counting efficiency for ^3H was 46%, determined by the internal standard method using a calibrated standard of ^3H-toluene (New England Nuclear).

(25R)-3β,26-Diacetoxycholest-5-ene (1b): (25R)-26-Hydroxycholesterol (1a, prepared from kryptogenin (11,12), 3.2 g, 8 mmol) was acetylated with a mixture of dry pyridine (dried over KOH) and acetic anhydride (125 ml) to provide 3.11 g (80%) of desired product (mp = 125-127°C, hexane-ether, reported (11,12) 128-129°C).

3β,26-Diacetoxycholest-5-en-7-one (2): A solution of diacetate 1b (2.38 g, 4.9 mmol) and mercuric bromide (2.64 g, 7.33 mmol) in 350 ml of t-butanol was irradiated with a Peh-Ray UV lamp for 25 hrs with stirring and a bubbling stream of dried air (~12 ml/min) (13). After removal of solvent and the usual work-up, 2.21 g of product were obtained, which were crystallized from hexane and from methanol to afford 2.17 g (89%) of enone 2 (mp = 118-120°C, reported (12) 121-122°C).

Catalytic Hydrogenation of (25R)-3β,26-diacetoxycholest-5-ene-7-one (2): The title compound (1.4 g, 2.8 mmol) dissolved in 150 ml of ethyl acetate was mixed with platinum dioxide (225 mg) and shaken with 2 atmospheres of hydrogen gas for 3 hrs in a Parr hydrogenation apparatus. The catalyst was removed (first by filtration and then through a disposable syringe filter, Acrodisc-CR, 0.45 μm, Gelman, Ann Harbor, MI), the solvent evaporated and after high-vacuum for 30 min., 1.23 g of mixture remained (recovery, 87%).

Separation of part of the mixture by semi-prep HPLC (μPorasil; CH_2Cl_2:hexane:MeOH/250:250:3) and further plc (2mm silica; hexane:ethyl acetate:methanol/200:40:4) showed that the mixture included 34% of the 7β-ol and traces of 7α-ol, 64% of the 5α-7-ketone, and 5α-cholestane-3β,26-diol diacetate (18) (4%).

Catalytic Reduction with Tritium Gas: This reaction was performed by New England Nuclear according to supplied directions. (25R)-3β,26-Diacetoxycholest-5-en-7-one (2, 25 mg, 50 μmol) dissolved in 5 ml of dry ethyl acetate was stirred in an atmosphere of 5 Ci of tritium with 80 mg of 5% Pd on carbon for 2 hrs at room temperature. After removal of labile tritium in vacuo with methanol and of the catalyst by filtration, the reaction products were dried in vacuo, and provided in 10 ml of ethanol (629 mCi, 25.0 mCi/mg). An aliquot (1.6 ml)

was diluted with a 25 ml ethereal solution of 100 mg of the mixture of compounds 3, 4 and 5 previously obtained in the catalytic hydrogenation of 2. By tlc (ethyl acetate: hexane/1:3) two spots could be detected with R_f's 0.13 and 0.30 corresponding to 7ξ(OH)- and 7-oxo derivatives (4 and 3, respectively); no UV absorbing material was detected. Another tlc plate developed as above was assayed for radioactivity, and found to contain 36% in the area of the 7ξ-ol (4), 61% as the 7-oxo derivative (3) and 3% as a nonpolar component (probably the 7-deoxy-5α-derivative (5), by analogy with products from the non-radioactive synthesis).

Lithium Aluminum Hydride Reduction of the Mixture of 3, 4 and 5: The radioactive mixture dissolved in 30 ml of dry ether was reduced with $LiAlH_4$ for 16 hrs at 0°C. Excess $LiAlH_4$ was destroyed with ethyl acetate, the precipitate was filtered through a short column of Celite, washed with a small amount of methanol and filtered again through a disposable syringe filter (Acrodisc-CR, 0.45 μm, Gelman, Ann Harbor, MI). After concentration of the solvent under dry nitrogen, tlc of a sample (2-propanol:hexane/15:85) showed two major spots, R_f's 0.21 and 0.23 (isomers of 6, by comparison with authentic standards) and a minor spot, R_f 0.34 (probably the 7-deoxy-diol, 7).

CrO_3 Oxidation of Compounds 6 and 7: The dried mixture from the above reduction was dissolved in 24 ml of acetone and titrated at 0°C with 500 μl of the Jones reagent (16). After stirring for 30 min. at 0°C and quenching with 1 ml of methanol, the solution was concentrated by a stream of N_2 and extracted with ether (20 ml). Tlc (2-propanol:hexane/15:85) showed one major spot, (R_f 0.45, corresponding to 8 with the standard), a minor spot at R_f 0.57 (compound 9) and a few traces of less polar material. The distribution of radioactivity was 44% in 8 and 32% in the area of 9.

This mixture was separated on two plc plates (2 mm thickness x 200 x 200 mm; 2-propanol:hexane/15:85) visualized by spraying with water, scraped, and extracted with 150 ml methylene chloride. The fraction containing 8 retained 38% of the tritium.

Methyl (25R)-[5α,6α-$^3H_2$]-3,7-dioxo-5α-cholestan-26-oate (10): Treatment of compound 8 in 1.35 ml of methanol with 2,2-dimethoxypropane (1.35 ml) and con-

centrated HCl (270 µl) afforded the ester 10; R_f 0.32 (hexane:ethyl acetate: acetic acid/110:37:3). Radioassay showed that 74% of the activity on the tlc plate was present in the methyl ester (12) R_f 0.32. The non-radioactive ester had the following properties: m.p.: 124-125°C (from hexane); NMR: δ (ppm) 0.67 (3H, s, C18), 0.90 (3H, d, J=6Hz, C21), 1.14 (3H, d, J=7Hz, C26), 1.25 (3H, s, C19), 1.80-2.60 (8H, m, C2+C4+C6+C25) and 3.67 (3H, s, CH_3 ester); IR: ν (cm^{-1}) 2940 and 2860 (aliph, C-H str.), 1739 (ester, C=O str.), 1723 and 1708 (two ketones, C=O str.), 1164 (ester, C-O str.); MS: (m/z) 444 (47%, M^+), 426 (9%, M-H_2O), 412 (18%, M-CH_3OH), 384 (17%, M-$H_2CO_2CH_3$), 287 (30%, M-side chain), 269 [42%, M-(side chain+ H_2O)], 260 [17%, M-(side chain+C16+C17)], 247, 246 and 245 [29%, 54% and 40%, M-side chain+C15+C16+C17 and H-migration)] and 192 (100%). GC: 3% OV-1, 260°, RRT 2.78, relative to methyldeoxycholate.

Methyl (25R)-[5α,6α-$^3H_2$]-3α,7α-dihydroxy-5α-cholestan-26-oate (11): Reduction of the above product simultaneously at positions C-3 and C-7 with K-Selectride at -45°C in a chlorobenzene-dry ice bath (13) provided product 11, with R_f 0.23, identical to the non-radioactive ester (toluene:acetone:methanol/ 160:60:1); 69% of the ^3H was present in the band of the diol 11. Purification by preparative tlc on a 1 mm-thick plate of silica provided an oil which resisted crystallization from acetone or from hexane. The non-radioactive diol was also well defined: NMR: δ (ppm) 0.65 (3H, s, C18), 0.78 (3H, s, C19), 0.88 (3H, d, J=6Hz, C21), 1.13 (3H, d, J=7Hz, C26), 3.67 (3H, s, CH_3 ester), 3.82 (1H, sharp m, β-H at C7) and 4.06 (1H, sharp m, β-H at C3). MS: 448 (7%, M^+), 430 (100%, M-H_2O), 415 [19%, M-(CH_3+H_2O)], 412 (48%, M-$2H_2O$), 397 [23%, M-($2H_2O$+CH_3)], 291 (9%, M-side chain), 273 [34%, M-(H_2O+side chain)], 255 [23%, M-($2H_2O$+side chain)], 249 (43%), 246 (43%), 231 (22%), 228 (33%) and 213 (45%). GC: (3% OV-1, RRT 2.19). The radioactive compound (11) was compared to it and found to be identical in tlc.

(25R)-[5α,6α-$^3H_2$]-3α,7α-Dihydroxy-5α-cholestan-26-oic acid (12): The oily methyl ester 11 was hydrolyzed in fresh 5% methanolic KOH. Distilled water (5 ml) was added and most of the methanol was evaporated under a stream of N_2. After the pH was brought to 3, the free acid was extracted with ether (3x3 ml), the ether phase was washed with water (3 ml), dried over $MgSO_4$, filtered and evaporated. The free acid 12 was crystallized from hot methanol (8), and had a

specific activity of 0.146 mCi/mg or 63.5 mCi/mmol with R_f 0.16 (hexane:ethyl acetate:acetic acid/15:14:1), identical to the non-radioactive acid. The physical properties of the acid have been reported (8).

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