STEREOSPECIFIC INTRODUCTION OF TRITIUM IN THE 16α OR 16β POSITION OF CHOLESTEROL

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SUMMARY

Reduction of cholest-5-en-3β-ol-16-one with KB^3H_4 afforded a mixture of [16α-^{3}H]-cholest-5-en-3β,16β-diol and [16β-^{3}H]-cholest-5-en-3β,16α-diol, which were separated and converted, respectively, into [16β-^{3}H]-cholest-5-en-3β-ol and [16α-^{3}H]-cholest-5-en-3β-ol.

In the biosynthetic pathways of many natural steroids the hydrogen atoms of the 16-position are involved. Therefore, a method for the synthesis of steroids labelled with tritium at C-16 is desirable.

This paper describes the stereospecific introduction of tritium label in 16β- or 16α- position of cholesterol, the general precursor of these compounds.

Our synthetic scheme is based on the reduction with KB^3H_4 in dioxane-isopropanol at room temperature of the ketonic group of cholest-5-en-3β-ol-16-one (2), which can be obtained from cholest-5-en-3β,

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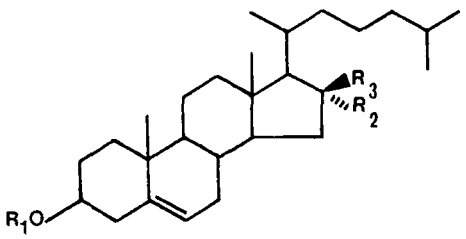
16β-diol (1) [1,2] either by direct selective oxidation to cholest--5-en-3β-ol-16-one (2) or by selective protection of the 3-OH group as benzoate (3) [2], followed by oxidation of the 16-OH group to give (4).



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1 R_1=R_2=H; R_3=OH

2 R_1=H; R_2R_3=O

3 R_1=COC_6H_5; R_2=H; R_3=OH

4 R_1=COC_6H_5; R_2R_3=O

5 R_1=R_3=H; R_2=OH

6 R_1=COC_6H_5; R_3=H; R_2=OH

7 R_1=COC_6H_5; R_2=H; R_3=OMs

8 R_1=R_2=R_3=H

10 R_1=$COCH_3$; R_2=R_3=H

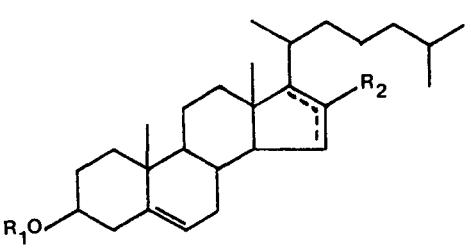
12 R_1=COC_6H_5; R_3=H; R_2=OTs

13 R_1=H; R_2=^{3}H; R_3=OH

14 R_1=H; R_2=OH; R_3=^{3}H

15 R_1=R_2=H; R_3=^{3}H

16 R_1=R_3=H; R_2=^{3}H



9 R_1=R_2=H

11 R_1=$COCH_3$; R_2=H

The reduction of the 16 ketonic group of (2) is a very slow process, requiring 96 hours to go to completion; nevertheless, the alkali often present in large amounts in commercial KB^3H_4 or NaB^3H_4

does not epimerize the 17-centre in a significant extent, as was shown by control experiments effected equilibrating a sample of (2) with sodium methoxide in methanol.

Treatment of the ketone (2) with KBH_4 afforded a (9:1) mixture of the known 16β-alcohol (1) and of its 16α- epimer (5), which were separated by preparative TLC.

Cholest-5-en-3β,16β-diol (1) was transformed into 3β-benzoyloxy--cholest-5-en-16β-ol (3) [2], which was treated with mesyl chloride in pyridine; the obtained 16β-mesylate (7) was directly transformed with $LiAlH_4$ in ethyl ether into the reduction product cholest-5-en--3β-ol (8) accompanied by ca. 20% of a second product (probably an elimination product (9)).

Cholest-5-en-3β-ol (8) was separated from the accompanying compound (9) by acetylation to (10) and (11), separation of the acetates by preparative TLC on silica gel-$AgNO_3$ and reconversion to (8) by treatment with $LiAlH_4$.

The transformation of cholest-5-en-3β,16α-diol (5) into cholest--5-en-3β-ol (8) was effected analogously working on the easily obtainable 16α-tosylate: treatment of cholest-5-en-3β,16α-diol (5) with benzoyl chloride in pyridine afforded the 3-monobenzoate (6), the structure of which was confirmed by comparison with the authentic compound obtained, as minor product, by reduction of 3β-benzoyloxy-cholest-5-en-16-one (4) with KBH_4; the 3-monobenzoate was tosylated with tosyl chloride in pyridine and the obtained 3β-benzoyloxy-16α-tosyloxycholest-5-ene (12) was transformed into cholest--5-en-3β-ol (8) with the same procedure described for the 16β-epimer.

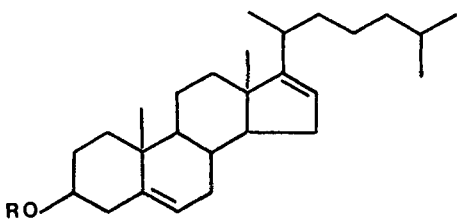
The above scheme was repeated to introduce the label: reduction of (2) with KB^3H_4 yielded a (9:1) mixture of [16α-^{3}H]-cholest-5-en--3β,16β-diol (13) and [16β-^{3}H]-cholest-5-en-3β-16α-diol (14), the first of which was transformed into [16β-^{3}H]-cholest-5-en-3β-ol (15) whereas the second was transformed into [16α-^{3}H]-cholest-5-en--3β-ol (16).

The 16β-orientation to the tritium atom of (15) and the 16α-orientation to the tritium atom of (16) were assigned on the basis of the

well known [3] inversion of configuration which occurs during the reduction of mesylates and tosylates with $LiAlH_4$.

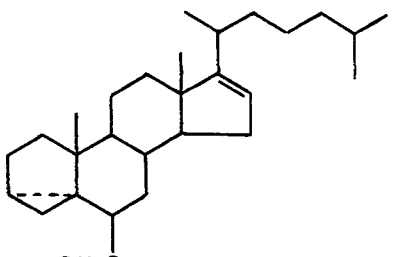
A higher activity at C-16 might be obtained by catalytic tritiation of a Δ^{16}-double bond.

Attempted regio- and selective hydrogenation with homogeneous catalyst of the Δ^{16}-double bond of 3β-benzoyloxy-cholesta-5,16-diene (17), prepared from 3β-benzoyloxy-cholest-5-en-16β-ol (3), was unsuccessful, since the compound was recovered unaltered after 24 hrs.



17 R=COC_6H_5 18 R=H

19 R=$COCH_3$



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We turned then to the $Pd/CaCO_3$ hydrogenation described by Berstein et al. [4] for the synthesis of [16,17-^{3}H]-cholest-5-en-3β--ol-22-one. To this aim the diene (17) was converted [2] into 6β--methoxy-3α,5-cyclo-5α-cholest-16-ene (20). As $Pd/CaCO_3$ catalysed hydrogenations can lead to scrambling, we preliminarly effected a deuteration of (20) with $^2H_2$ on $Pd/CaCO_3$: mass spectral analysis of the deuterated sample showed extensive scrambling, making this process unreliable for a stereospecific synthesis, but still useful to introduce a high activity. In fact catalytic tritiation on $Pd/CaCO_3$ followed by hydrolysis [5], afforded tritiated cholest-5-en--3β-ol which was > 95% chemically and radiochemically pure (specific activity 1.05×10^{10} dpm of ^3H/mg).

EXPERIMENTAL

All m.ps. were uncorrected. IR spectra were recorded in nujol, unless otherwise stated, using a Perkin-Elmer 237 spectrophotometer. NMR spectra were obtained using a Perkin-Elmer R12 (60 MHz) spectro

meter in $CDCl_3$ solvent, with TMS as internal reference and optical rotations were obtained using a Perkin-Elmer 141 Polarimeter as chloroform solutions. Elemental analyses were consistent with the calculated values. Preparative and analytical thin layer chromatographies (TLC) were carried out on Merck HF_{254} silica gel plates (0.25 mm); the products were detected by spraying with aqueous sulfuric acid. Radioactive samples were counted on a Packard Tri-Carb 3320 liquid scintillation counter.

Cholest-5-en-3β-ol-16-one (2)

Cholest-5-en-3β,16β-diol (1) (10 g) was dissolved into a solution of 55.8 g of sodium acetate in 1100 ml of glacial acetic acid. To this solution was slowly added a solution of 1.8 g of CrO_3 in 160 ml of water and 36 ml of CH_3COOH under stirring at r.t.; the reaction mixture was left at r.t. for 16 hrs, then poured into ice-water and extracted with $CHCl_3$. The organic extract was washed with 5% Na_2CO_3 solution, water and dried under Na_2SO_4. Evaporation of the solvent gave 8 g of crude material which was purified by column chromatography (silica-gel HR 60; eluent: benzene-ethyl acetate 6:4). The eluted compound was crystallized from methanol to yield 3.6 g of pure (2), m.p. 155-56°C; $[\alpha]_D^{20}$ = -171°; ν max: 3400, 1735 cm^{-1}; NMR: 0.82 (s, 18-CH_3), 0.87 (d, J=7Hz, 26 and 27 CH_3's), 0.99 (d, J=7Hz, 21-CH_3), 1.1 (s, 19-CH_3), 3.50 (m, 3α-H), 5.35 (m, 6-H) δ.

3β-Acetate: m.p. (methanol) 107°C; $[\alpha]_D^{20}$ = -160°.

3β-benzoyloxycholest-5-en-16β-ol (3)

Cholest-5-en-3β,16β-diol (1) (4 g) was submitted to benzoylation with benzoyl chloride/pyridine as described [2] and the crude product was chromatographed on silica-gel/celite (1:1; w/w). Elution with pentane/benzene (1:1) afforded 3.1 g of 3β-benzoyloxycholest-5-en-16β-ol (3), which was crystallized from methanol, m.p. 181-185°, $[\alpha]_D^{20}$ = -10°; ν max: 3600, 1710, 1605, 1585, 1280 cm^{-1}; NMR: 0.81 (d, J=7Hz, 26 and 27 CH_3's), 0.82 (s, 18-CH_3), 0.93 (d, J=7Hz, 21-CH_3), 1.00 (s, 19-CH_3), 4.3 (m, 16α-H), 4.8 (m, 3α-H), 5.4 (m, 6-H), 7.5-8.2 (m, C_6H_5COO-) δ.

3β-benzoyloxycholest-5-en-16-one (4)

3β-benzoyloxycholest-5-en-16β-ol (3) (3 g) in 85 ml of freshly distilled DMF was treated with a solution of 2.37 g of CrO_3 and 60 ml of 1% H_2SO_4, in DMF; after 15 minutes at r.t., the mixture was poured into ice-water. The solid was filtered off, washed and dried. Recrystallisation from methanol gave 2.5 g of pure (4): m.p. 168--170°C; $[\alpha]_D^{20} = -114°$; ν max (KBr): 1730, 1715, 1275 cm^{-1}; NMR: 0.85 (s, 18-CH_3), 0.88 (d, J=7Hz, 26 and 27 CH_3's), 1.00 (d, J=7Hz, 21--CH_3), 1.1 (s, 19-CH_3), 4.89 (m, 3α-H), 5.45 (m, 6-H), 7.25-8.20 (m, C_6H_5COO-) δ.

Equilibration of (2)

A solution of 250 mg of (2) into 50 ml of 1N CH_3ONa/CH_3OH was refluxed for 72 hrs under nitrogen atmosphere; the product, recovered by addition of diluted HCl and usual work-up, was found chromatographically homogeneous by TLC in two systems (benzene-ethyl acetate 6:4 (v/v); n-hexane-chloroform-acetone 7:3:1 (v/v)) and VPC (as benzoyl derivative, SE-30 2.5%, T_c = 280°C); after crystallization from CH_3OH it was proved to be identical (m.p., ν max, $[\alpha]_D^{20}$, NMR) to a pure sample of (2).

3β-benzoyloxycholest-5-en-16β-ol (3) and 3β-benzoyloxycholest-5-en--16α-ol (6)

To a solution of 1 g of (4) into 50 ml of isopropyl alcohol-dioxane 1:1 (v/v), was added 1 g of KBH_4. After stirring for 3 days, the solvent was evaporated and the residue was partitioned between HCl/H_2O (10%) and ethyl ether. The ethereal layer was separated and the aqueous phase was twice extracted with ether. The ether layer was washed with saturated brine and dried on Na_2SO_4. Evaporation of the solvent in vacuo yielded 1 g of crude material, which was chromatographed on SiO_2 HR 60 (50 g) (eluent: n-hexane-acetone 20:1 (v/v)). The 16β-hydroxy-derivative (600 mg), eluted first and crystallyzed from methanol, was identifical to the product obtained by monobenzoylation of (1). The same eluent eluted successively 3β-benzoyloxycholest-5-en-16α-ol (6) (150 mg), which was crystallized from methanol, m.p. 161-163°C; $[\alpha]_D^{20} = -17°$ ($CHCl_3$); ν max

(KBr): 3200, 3060, 2030, 1605, 1585 cm^{-1}; NMR: 0.68 (s, 18-CH_3), 0.82 (d, J=7Hz, 26 and 27 CH_3's), 0.92 (d, J=6Hz, 21-CH_3), 1.03 (s, 19-CH_3), 4.1 (m, 16β-H), 4.8 (m, 3α-H), 5.4 (m, 6-H), 7.5-8.2 (m, C_6H_5COO-) δ.

Cholest-5-en-3β,16β-diol (1) and cholest-5-en-3β,16α-diol (5)

To a solution of 500 mg of (2) into 10 ml of isopropyl alcohol--dioxane 1:1, 500 mg of KBH_4 were added under stirring. After 96 hrs, work-up as described above yielded 490 mg of crude product. Preparative TLC (benzene-hexane-acetone 4.5:4.5:1, three elutions) afforded 430 mg of higher R_f cholest-5-en-3β,16β-diol (1) [2], and 45 mg of lower R_f cholest-5-en-3β,16α-diol (5), which was crystallized from methanol, m.p. 175-177°C; $[\alpha]_D^{20} = -37°$; ν max: 3500-3300 (broad band) cm^{-1}; NMR: 0.65 (s, 18-CH_3), 0.79 (d, J=7Hz, 26 and 27 CH_3's), 0.88 (d, J=7Hz, 21-CH_3), 0.92 (s, 19-CH_3), 3.4 (m, 3α-H), 4.1 (m, 16β-H), 5.4 (m, 6-H) δ.

Cholest-5-en-3β-ol (8) from cholest-5-en-3β,16β-diol (1)

A solution of 200 mg of (3), obtained from (1) as described [2], into 4 ml of dry pyridine was cooled at 0° and 0.4 ml of methanesulfonyl chloride were added. The mixture was allowed to stand at 0° for 24 hrs, after which ice-water was added and the product extracted with ethyl ether. The organic layer was washed with water, 2N HCl, water and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* afforded 230 mg of an oil, which was dissolved into dry ether and directly submitted to reduction with an excess of $LiAlH_4$. After 16 hrs at r.t. with stirring, usual work-up yielded 142 mg of crude product. Acetylation with Ac_2O/pyridine and preparative TLC (pentane-benzene 1:1 as eluent, two elutions) on 20% $AgNO_3$-silica gel afforded 100 mg of product identical to 3β-acetoxycholest-5-ene (10) and 16 mg of a more polar product (presumably an insaturated compound (11) which was no further investigated). (10) was transformed into (8) by reduction with $LiAlH_4$ in ether as described above.

Cholest-5-en-3β-ol (8) from cholest-5-en-3β,16α-diol (5)

Cholest-5-en-3β,16α-diol (5) (45 mg) was transformed into 3β-benzoyloxycholest-5-en-16α-ol (6) (37 mg) as described for the 16β-epi

mer. The product was identical (mixed m.p., $[\alpha]_D^{20}$; ν max, NMR) to the minor product obtained (together with the predominant 16β-alcohol (3)) from the reduction of 3β-benzoyloxy-cholest-5-en-16-one (4). To a solution of 200 mg of (6) into 4 ml of dry pyridine 300 mg of p-toluenesulfonyl chloride were added. The reaction mixture was left at r.t. for 36 hrs, after which usual work-up afforded an oil, which was reduced with $LiAlH_4$ in ether as described for (7). The recovered product (138 mg) was submitted to acetylation and purified by preparative TLC (pentane-benzene 1:1, 2 elutions), to yield 103 mg of 3β-acetoxycholest-5-ene (10) which was transformed into cholest-5-en-3β-ol (8) by reduction with $LiAlH_4$ as described above.

[16α-^{3}H]-cholest-5-en-3β,16β-diol (13) and [16β-^{3}H]-cholest-5-en-3β,16α-diol (14)

Cholest-5-en-3β-ol-16-one (2) (20 mg) was dissolved into 4 ml of dioxane-isopropyl alcohol (1:1) and KB^3H_4 (400 mCi, specific activity 20 Ci/mM) was added. After 96 hrs at r.t. under stirring the products were recovered as described for (1) and (5), and 3 mg of carrier (1) and 3 mg of carrier (5) were added. Separation by preparative TLC (hexane-chloroform-acetone 14:6:3, three elutions) afforded chemically and radiochemically pure [16α-^{3}H]-cholest-5-en-3β,16β-diol (13) (2.9×10^9 dpm of ^{3}H/mg) and [16β-^{3}H]-cholest-5-en-3β,16α-diol (14) (8.1×10^8 dpm of ^{3}H/mg).

[16β-^{3}H]-cholest-5-en-3β-ol (15)

(13) was transformed into (15) as described for the conversion of the non radioactive 16β-alcohol (1) into (8).

[16β-^{3}H]-cholest-5-en-3β-ol (15), which was found to be chemically and radiochemically pure, exhibited a total activity of 6.64×10^8 dpm of ^{3}H.

[16α-^{3}H]-cholest-5-en-3β-ol (16)

(16), chemically and radiochemically pure (total activity of 6.11×10^7 dpm of ^{3}H) was obtained from [16β-^{3}H]-cholest-5-en-3β,16α-diol (14) as described for the non radioactive material (5).

3β-benzoyloxycholest-5,16-diene (17)

A solution of 1.4 g of (3) and 1.05 g of p-toluenesulfonyl chloride in 14 ml of dry pyridine was left at 40°C for 38 hrs. The solution was slowly poured into an ice cold 5% $KHCO_3$ solution and extracted with ethyl acetate. The extract was washed with 3% HCl, 5% $KHCO_3$ solutions, saturated brine and dried over $NaSO_4$. Solvent evaporation gave 1.2 g of crude material, which was submitted to column chromatography (silica gel HR 60; eluent: isooctane-acetone 95:5 v/v), which afforded 450 mg of (17). An analytical sample was obtained by preparative TLC and crystallisation from methanol: m.p. 131-132°C; $[\alpha]_D^{20} = -25°$; ν max: 1710, 1280, 1585, 1605, 1625 cm^{-1}; NMR: 0.78 (s, 18-CH_3), 0.84 (d, J=7Hz, 26 and 27 CH_3's), 0.97 (d, J=7Hz, 21-CH_3), 1.10 (s, 19-CH_3), 5.25 (m, 16-H), 5.4 (m, 6-H), 7.3-8.2 (m, C_6H_5COO-) δ.

3β-hydroxycholest-5,16-diene (18)

The benzoate (17) (100 mg) was hydrolyzed with 3% Na_2CO_3 and crystallized from methanol: m.p. 168-170°C (Lit. [6]: 167-170°C). The 3-acetate (19) of (18) had m.p. (CH_3OH) 79.5-81°C; $[\alpha]_D^{20} = -58.5°$; ν max: 3055, 1740, 1625, 1240 cm^{-1}; NMR: 0.77 (s, 18-CH_3), 0.83 (d, J=7Hz, 26 and 27 CH_3's), 0.95 (d, J=7Hz, 21-CH_3), 1.02 (s, 19-CH_3), 1.98 (s, CH_3COO-), 5.25 (m, 16-H), 5.4 (m, 6-H).

[16.17-^{3}H]-cholest-5-en-3β-ol

6β-Methoxy-3α,5-cyclo-5α-cholest-16-ene (20) (5 mg), obtained from (18) according to Chaudhuri et al. [2], was dissolved into 1 ml of ethyl acetate and 2.5 mg of $Pd/CaCO_3$ were added; hydrogenation with tritium gas (2.59 Ci/ml) for 1 hr yielded a mixture of the tritiated product and of the starting compound, which were separated by 20% $AgNO_3$-silica gel TLC (pentane-benzene 1:1).

The tritiated product (total activity 2.02×10^{10} dpm of ^{3}H) was dissolved into 0.5 ml of freshly distilled dioxane and 0.5 ml of water. After addition of 1 mg of p-toluenesulfonic acid, the mixture was left at 75-80° for 6 hrs. Evaporation of the solvent, partition between benzene and water and evaporation of the organic layer after

drying over Na_2SO_4 yielded [16,17-$^3H_2$]-cholest-5-en-3β-ol which was purified by preparative TLC on silica gel (benzene-ethyl acetate 7:3 as eluent) and exhibited a specific activity of 1.05×10^{10} dpm of ^3H/mg.

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