

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

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SUMMARY

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

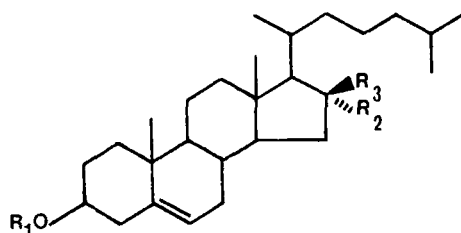
This paper describes the stereospecific introduction of tritium label in 16 β - or 16 α - position of cholesterol, the general precursor of these compounds.

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-3 β -ol-16-one (2), which can be obtained from cholest-5-en-3 β ,

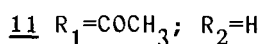
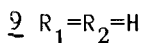
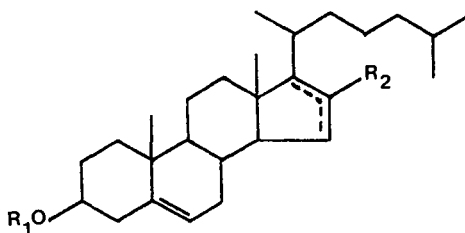
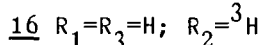
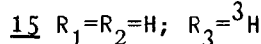
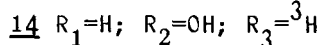
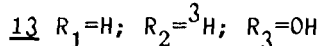
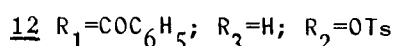
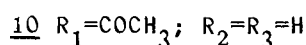
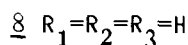
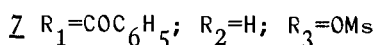
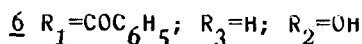
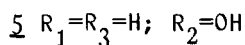
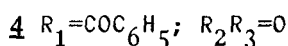
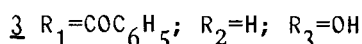
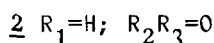
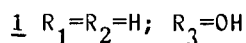
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16 β -diol (1) (1,2) either by direct selective oxidation to cholest-5-en-3 β -ol-16-one (2) or by selective protection of the 3-OH group as benzoate (3) (2), followed by oxidation of the 16-OH group to give (4).



1



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 16 β -alcohol (1) and of its 16 α -epimer (5), which were separated by preparative TLC.

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

Cholest-5-en-3 β -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-3 β ,16 α -diol (5) into cholest-5-en-3 β -ol (8) was effected analogously working on the easily obtainable 16 α -tosylate: treatment of cholest-5-en-3 β ,16 α -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 3 β -benzoyloxy-cholest-5-en-16-one (4) with KBH_4 ; the 3-monobenzoate was tosylated with tosyl chloride in pyridine and the obtained 3 β -benzoyloxy-16 α -tosyloxycholest-5-ene (12) was transformed into cholest-5-en-3 β -ol (8) with the same procedure described for the 16 β -epimer.

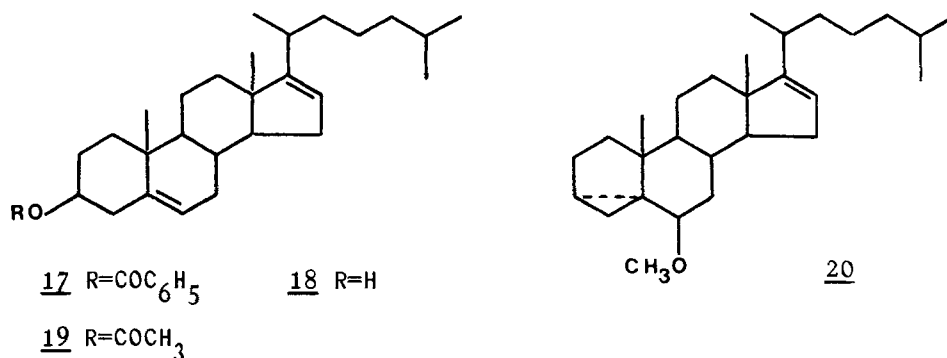
The above scheme was repeated to introduce the label: reduction of (2) with KB^3H_4 yielded a (9:1) mixture of $[16\alpha\text{-}^3\text{H}]$ -cholest-5-en-3 β ,16 β -diol (13) and $[16\beta\text{-}^3\text{H}]$ -cholest-5-en-3 β -16 α -diol (14), the first of which was transformed into $[16\beta\text{-}^3\text{H}]$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

The 16 β -orientation to the tritium atom of (15) and the 16 α -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-16 β -ol (3), was unsuccessful, since the compound was recovered unaltered after 24 hrs.



We turned then to the Pd/CaCO_3 hydrogenation described by Bernstein et al. (4) for the synthesis of $[16,17\text{-}^3\text{H}]$ -cholest-5-en-3 β -ol-22-one. To this aim the diene (17) was converted (2) into 6 β -methoxy-3 α ,5-cyclo-5 α -cholest-16-ene (20). As Pd/CaCO_3 catalysed hydrogenations can lead to scrambling, we preliminarily effected a deuteration of (20) with $^2\text{H}_2$ 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-3 β -ol which was $> 95\%$ chemically and radiochemically pure (specific activity 1.05×10^{10} dpm of $^3\text{H}/\text{mg}$).

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-3 β -ol-16-one (2)

Cholest-5-en-3 β ,16 β -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; $[\alpha]_{\text{D}}^{20} = -171^\circ$; ν max: 3400, 1735 cm^{-1} ; NMR: 0.82 (s, 18- CH_3), 0.87 (d, $J=7\text{Hz}$, 26 and 27 CH_3 's), 0.99 (d, $J=7\text{Hz}$, 21- CH_3), 1.1 (s, 19- CH_3), 3.50 (m, 3 α -H), 5.35 (m, 6-H) δ .

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

3 β -benzoyloxycholest-5-en-16 β -ol (3)

Cholest-5-en-3 β ,16 β -diol (1) (4 g) was submitted to benzylation 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 3 β -benzoyloxycholest-5-en-16 β -ol (3), which was crystallized from methanol, m.p. 181-185°, $[\alpha]_{\text{D}}^{20} = -10^\circ$; ν max: 3600, 1710, 1605, 1585, 1280 cm^{-1} ; NMR: 0.81 (d, $J=7\text{Hz}$, 26 and 27 CH_3 's), 0.82 (s, 18- CH_3), 0.93 (d, $J=7\text{Hz}$, 21- CH_3), 1.00 (s, 19- CH_3), 4.3 (m, 16 α -H), 4.8 (m, 3 α -H), 5.4 (m, 6-H), 7.5-8.2 (m, $\text{C}_6\text{H}_5\text{COO-}$) δ .

3 β -benzoyloxycholest-5-en-16-one (4)

3 β -benzoyloxycholest-5-en-16 β -ol (3) (3 g) in 85 ml of freshly distilled DMF was treated with a solution of 2.37 g of CrO₃ and 60 ml of 1% H₂SO₄ 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; $[\alpha]_D^{20} = -114^\circ$; ν_{max} (KBr): 1730, 1715, 1275 cm⁻¹; NMR: 0.85 (s, 18-CH₃), 0.88 (d, J=7Hz, 26 and 27 CH₃'s), 1.00 (d, J=7Hz, 21-CH₃), 1.1 (s, 19-CH₃), 4.89 (m, 3 α -H), 5.45 (m, 6-H), 7.25-8.20 (m, C₆H₅COO-) δ .

Equilibration of (2)

A solution of 250 mg of (2) into 50 ml of 1N CH₃ONa/CH₃OH 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 CH₃OH it was proved to be identical (m.p., ν_{max} , $[\alpha]_D^{20}$, NMR) to a pure sample of (2).

3 β -benzoyloxycholest-5-en-16 β -ol (3) and 3 β -benzoyloxycholest-5-en-16 α -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 KBH₄. After stirring for 3 days, the solvent was evaporated and the residue was partitioned between HCl/H₂O (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 Na₂SO₄. Evaporation of the solvent in vacuo yielded 1 g of crude material, which was chromatographed on SiO₂ HR 60 (50 g) (eluent: n-hexane-acetone 20:1 (v/v)). The 16 β -hydroxy-derivative (600 mg), eluted first and crystallized from methanol, was identical to the product obtained by monobenzoylation of (1). The same eluent eluted successive-

ly 3 β -benzoyloxycholest-5-en-16 α -ol (6) (150 mg), which was crystallized from methanol, m.p. 161-163°C; $[\alpha]_D^{20} = -17^\circ$ (CHCl₃); ν_{max}

(KBr): 3200, 3060, 2030, 1605, 1585 cm^{-1} ; NMR: 0.68 (s, 18- CH_3), 0.82 (d, $J=7\text{Hz}$, 26 and 27 CH_3 's), 0.92 (d, $J=6\text{Hz}$, 21- CH_3), 1.03 (s, 19- CH_3), 4.1 (m, 16 β -H), 4.8 (m, 3 α -H), 5.4 (m, 6-H), 7.5-8.2 (m, $\text{C}_6\text{H}_5\text{COO-}$) δ .

Cholest-5-en-3 β ,16 β -diol (1) and cholest-5-en-3 β ,16 α -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-3 β ,16 β -diol (1) (2), and 45 mg of lower R_f cholest-5-en-3 β ,16 α -diol (5), which was crystallized from methanol, m.p. 175-177°C; $[\alpha]_D^{20} = -37^\circ$; ν_{max} : 3500-3300 (broad band) cm^{-1} ; NMR: 0.65 (s, 18- CH_3), 0.79 (d, $J=7\text{Hz}$, 26 and 27 CH_3 's), 0.88 (d, $J=7\text{Hz}$, 21- CH_3), 0.92 (s, 19- CH_3), 3.4 (m, 3 α -H), 4.1 (m, 16 β -H), 5.4 (m, 6-H) δ .

Cholest-5-en-3 β -ol (8) from cholest-5-en-3 β ,16 β -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 3 β -acetoxysterol (10) and 16 mg of a more polar product (presumably an unsaturated 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-3 β -ol (8) from cholest-5-en-3 β ,16 α -diol (5)

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

mer. The product was identical (mixed m.p., $[\alpha]_D^{20}$; η max, NMR) to the minor product obtained (together with the predominant 16 β -alcohol (3)) from the reduction of 3 β -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 LiAlH₄ 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 3 β -acetoxycholest-5-ene (10) which was transformed into cholest-5-en-3 β -ol (8) by reduction with LiAlH₄ as described above.

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

Cholest-5-en-3 β -ol-16-one (2) (20 mg) was dissolved into 4 ml of dioxane-isopropyl alcohol (1:1) and KB³H₄ (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 [16 α -³H]-cholest-5-en-3 β ,16 β -diol (13) (2.9×10^9 dpm of ³H/mg) and [16 β -³H]-cholest-5-en-3 β ,16 α -diol (14) (8.1×10^8 dpm of ³H/mg).

[16 β -³H]-cholest-5-en-3 β -ol (15)

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

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

[16 α -³H]-cholest-5-en-3 β -ol (16)

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

3 β -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; $[\alpha]_D^{20} = -25^\circ$; ν max: 1710, 1280, 1585, 1605, 1625 cm^{-1} ; NMR: 0.78 (s, 18- CH_3), 0.84 (d, $J=7\text{Hz}$, 26 and 27 CH_3 's), 0.97 (d, $J=7\text{Hz}$, 21- CH_3), 1.10 (s, 19- CH_3), 5.25 (m, 16-H), 5.4 (m, 6-H), 7.3-8.2 (m, $\text{C}_6\text{H}_5\text{COO-}$) δ .

3 β -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; $[\alpha]_D^{20} = -58.5^\circ$; ν max: 3055, 1740, 1625, 1240 cm^{-1} ; NMR: 0.77 (s, 18- CH_3), 0.83 (d, $J=7\text{Hz}$, 26 and 27 CH_3 's), 0.95 (d, $J=7\text{Hz}$, 21- CH_3), 1.02 (s, 19- CH_3), 1.98 (s, $\text{CH}_3\text{COO-}$), 5.25 (m, 16-H), 5.4 (m, 6-H).

[16,17- ^3H]-cholest-5-en-3 β -ol

6 β -Methoxy-3 α ,5-cyclo-5 α -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 ^3H) 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 $[16,17-^3\text{H}_2]$ -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 $^3\text{H}/\text{mg}$.

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