REGIO-CONTROLLED SYNTHESIS OF 4α-($^3H_3$)METHYL-5α-CHOLESTAN-3β-OL

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SUMMARY

4α-($^3H_3$)Methyl-5α-cholestan-3β-ol was regioselectively prepared from 5α-cholest-1-en-3-one via alkylation with methyl iodide, hydrogenation, and LAH reduction. Details of this synthesis are given. The final and intermediate products were characterised by spectroscopic methods.

Key Words: Regio-controlled synthesis, 4α-($^3H_3$)Methyl-5α-cholestan-3β-ol, 5α-Cholest-1,3-dien-3-ol trimethylsilyl ether.

INTRODUCTION

Recently, we reported the first mechanism-based inactivators of 4-methylsterol oxidase,[1] a microsomal enzyme system involved in cholesterol biosynthesis in rat liver.[2] Our continuing program required an assay utilizing the release of tritium (^3H) from the 4-methyl group for the evaluation of potential inhibitors. The published methods for making radiolabelled 4α-methyl-5α-sterols were not entirely satisfactory as they afforded regio-isomers that necessitated extensive and often tedious separation steps.[3] This paper describes a regio-controlled and highly stereoselective synthesis of 4α-methyl-5α-cholestan-3β-ol and the application of the procedure to the synthesis of 4α-($^3H_3$)methyl-5α-cholestan-3β-ol.

RESULTS AND DISCUSSION

2α-Methyl-5α-cholestan-3-one is the main product obtained when 5α-cholestan-3-one is sequentially treated with base and methyl iodide.[4] Improved selectivity towards C(4)-alkylation has been reported with 5α-cholest-7-en-3-one.[3] However the purification necessary to isolate the desired 4α-methylsterol made this compound unattractive to us as a precursor. We envisaged a synthetic route, Fig. 1, using an olefinic linkage at C(1) to

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block the reaction at C(2) and thereby direct alkylation to C(4).

5α-Cholest-1-en-3-one (1) was accordingly prepared by literature procedures,[5,6] and was deprotonated with lithium diisopropylamine (LDA). Trapping of the resulting enolate with chlorotrimethylsilane furnished 5α-cholest-1,3-dien-3-ol trimethylsilyl ether (2). The lithium enolate was conveniently regenerated with methyl lithium and reaction with methyl iodide, in the presence of a molar equivalent of hexamethylphosphoramide (HMPA) afforded 4α-methyl-5α-cholest-1-en-3-one. Saturation of the double bond was accomplished by 5% Pd/C catalysed hydrogenation. The product, 4α-methyl-5α-cholestan-3-one, was reduced with lithium aluminum hydride (LAH) at -78°C in ether to give a mixture of isomeric 3α- and 3β-alcohols These were separated by preparative high pressure liquid chromatography (hplc).

For the synthesis of 4α-($^3H_3$)methyl-5α-cholest-1-en-3-one (3), tritium labelled ($^3H_3$)methyl iodide was used and the reaction carried out in an exactly analogous manner. Yield was estimated by comparison of hplc chromatograms of labelled compound and authentic 4α-methyl-5α-cholest-1-en-

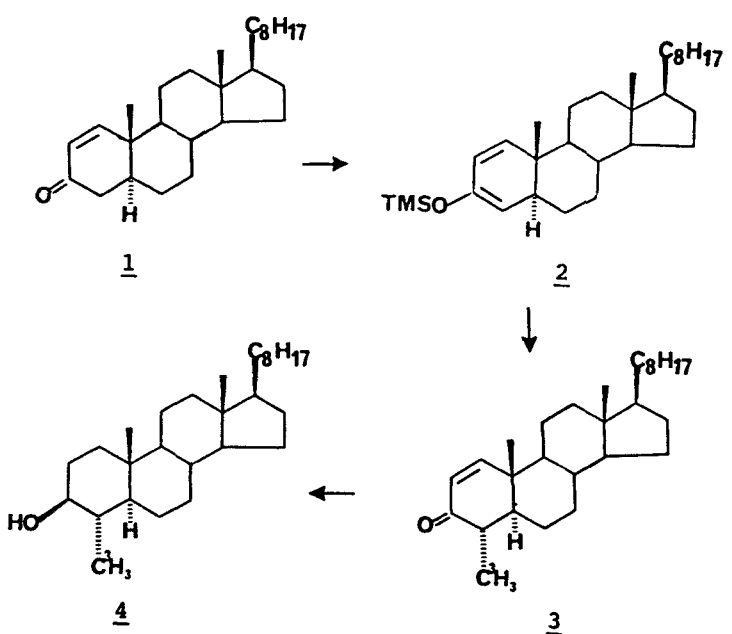

FIGURE 1

-3-one. Sequential catalytic hydrogenation and LAH reduction yielded the title compound (4). The yield was significantly lower when ($^3H_3$)methyl iodide was used (50%) than when unlabeled methyl iodide was employed (91%). The reaction with labelled methyl iodide was run under experimental conditions designed to mimic those used with unlabelled compound. The only experimental difference is that the labelled reaction was run in the original ampule which contained a small copper turning.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. ^1H NMR spectra were recorded in $CDCl_3$ with an IBM FT (80 MHz) spectrometer, IR spectra were recorded on a Perkin-Elmer 462, and UV spectra were obtained on Perkin-Elmer Lambda 3 instrument. Mass spectra were obtained on an LKB-6000 spectrometer. High pressure liquid chromatographic separations were performed on a Waters Associates Model 6000 instrument; a Waters semi-preparative μ-Porasil column was used in normal phase separation. ($^3H_3$)Methyl iodide was purchased from New England Nuclear.

5α-Cholest-1,3-dien-3-ol trimethylsilyl ether (2). To a solution of diisopropylamine (2.624 ml;18.74 mmole) in dry THF (39 ml) at -20°C and in an atmosphere of argon, was added n-butyl lithium (12.49 ml; 1.55 M solution in hexane). After 30 min, the temperature was lowered to -78°C and a solution of 5α-cholest-1-en-3-one [(1); 6.0 g; 15.6 mmole] in dry THF (15.6 ml) was added. The reaction mixture was stirred at 0°C for 1 hr and then cooled to -78°C.

Trimethylsilyl chloride (freshly distilled from K_2CO_3; 2.37 ml; 18.74 mmole) was added dropwise, and the mixture was stirred for 10 min and allowed to warm to room temperature over 1 hr. Hexane (300 ml) was added and the solution was then washed with saturated $NaHCO_3$ (2 x 400 ml), brine and dried over K_2CO_3. Solvent was removed *in vacuo* and product crystallized from acetone to afford 5α-cholest-1,3-dien-3-ol trimethylsilyl ether (5.02 g; 70.4%) mp 76-77°C. The mass spectrum showed a molecular ion at m/z 456 and other ions at m/z 441, 384, 206, 194. NMR: δ ($CDCl_3$) 6.02(1H,d,J=9.7Hz);

5.58(1H,dd,J=9.7,2 Hz); 4.53(1H,dd,J=2 Hz). I.R. ν max($CHCl_3$) 1635 cm^{-1}, UV (hexane) λ max 212 nm,(ε 2800); 275 nm,(ε 2300).

4α-Methyl-5α-cholest-1-en-3-one (3). Methyl lithium [193.5 μl (1.55 M in ether)] was added to a solution of 5α-cholest-1,3-dien-3-ol trimethylsilyl ether (136.8 mg; 0.3 mmole) in dry THF (1.0 ml) at -20°C under an atmosphere of argon. After 1 hr at 0°C, the temperature was lowered to -78°C and HMPA (43 μl; 0.3 mmole) was added. It was syringed into a pre-cooled (-78°C) ampule containing a solution of methyl iodide (15.5 μl; 0.25 mmole), in dry THF (0.5 ml) under argon. It was left to stand at room temperature overnight.

Excess volatile reagents and solvent were removed under a stream of argon and collected in cold traps. The residue was applied to a pipette column of neutral alumina (activity 11) 1.0 g, and eluted with 25% ethyl acetate in hexane (15 ml). The solvent was removed, the solid obtained was dissolved in hexane (2.0 ml) and purified by normal phase hplc (5% ethyl acetate in hexane). Crystallization from acetone gave 4α-methyl-5α-cholest-1-en-3-one (90 mg; 91%) mp 84-85°(Lit[4] 82-83°). The mass spectrum showed molecular ion at m/z 398, and others at m/z 383, 342, 300, 285, 275, 258 and 243. NMR ($CDCl_3$, 80 MHz), 7.11(1H,d,J=10Hz); 5.86(d,J=10Hz); 1.12(3,d,J=5.6Hz, 4-CH_3). IR ($CHCl_3$) ν max 1690 and 1660cm^{-1}. UV (hexane) λ max 222 nm, (ε 9500).

4α-($^3H_3$)Methyl-5α-cholest-1-en-3-one. Using tritium-labelled methyl iodide (C^3H_3I, 25 mCi, specific activity 97 mCi/mM) the radiosynthesis was carried out in exactly the procedure described above. The product was characterized by co-chromatography on tlc and hplc comparison with authentic 4α-methyl-5α-cholest-1-en-3-one. Estimated (hplc) yield was 50.2 mg, 50%.

4α-Methyl-5α-cholestan-3β-ol (4). 4α-Methyl-5α-cholest-1-en-3-one (72 mg) was placed in a flask equipped with a magnetic stirrer and a three-way adaptor connected to a vacuum pump and a balloon filled with hydrogen. Cyclohexane-THF [(3:2); 10 ml] and 5% Pd/C (20 mg) were added. The system was evacuated and then filled with hydrogen from the balloon. It was stirred under positive hydrogen pressure for 4 hr and filtered through a short pipette column of celite eluted with hexane (20 ml).

4α-Methyl-5α-cholestan-3-one, obtained from this sequence was immediately reduced with lithium aluminium hydride (20 mg) in dry ether (20 ml) at -78°C for 40 min. Excess LAH was destroyed with ethyl acetate, followed by dropwise addition of saturated Na_2SO_4 to coagulate the aluminate. It was dried, filtered, and the solvent was removed to dryness. The product was dissolved in chloroform (2.0 ml) and separated by normal phase hplc (15% ethyl acetate in hexane) into 4α-methyl-5α-cholestan-3α-ol (22 mg)[7] and 4α-methyl-5α-cholestan-3β-ol (51 mg). Crystallization from methanol gave 4α-methyl-5α-cholestan-3β-ol (50 mg; 69%), mp 163-164° (Lit[4] 164-165°). The mass spectrum showed molecular ion at m/z 402 and others at m/z 387, 384, 369, 250, 237. NMR: δ ($CDCl_3$) 3.05(1H,m,J=9.5,4.8Hz 3α-H); 0.93(1H,d,J=6.4Hz, 4-CH_3) IR ν max ($CHCl_3$), 3600cm^{-1}.

4α-($^3H_3$)Methyl-5α-Cholestan-3β-ol. Compound (3) was carried through the sequence of catalytic hydrogenation and reduction with LAH as described. The desired product (4) was characterized by co-chromatography (tlc and hplc) with authentic 4α-methyland 4α-[$^2H_3$]methyl-5α-cholestan-3β-ol.[8] It was recrystallized to constant specific activity (81.6 mCi/mM). This specific activity is somewhat lower than that (97mCi/mM) quoted by the manufacturers for the ($^3H_3$)methyl iodide which was used. This discrepancy may well be due to a lower actual value for the ($^3H_3$)methyl iodide, as we ourselves made no measurements on it.

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7. Additional 4α-Methyl-5α-cholestan-3β-ol is obtainable from the 4α-methyl-5α-cholestan-3α-ol via Collins oxidation and reduction.

8. 4α-($^{2}H_3$)Methyl-5α-cholestan-3β-ol was made from 4α-carbomethoxy-5α-cholest-1-en-3-one via 5% Pd/C catalyzed hydrogenation, $NaBH_4$ reduction, tetrahydropyranylation, LAD reduction, mesylation, and LAD reductive displacement of the mesylate, followed by removal of the 3-tetrahydropyranyl ether grouping.