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Preparation of 7α- and 7β-methylcholestane derivatives by kinetic separation of the diastereomeric mixture

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Abstract

7α- and 7β-Methyl derivatives of cholest-5-en-3β-ol (cholesterol), cholest-4-en-3-one and cholest-4-en-3,6-dione were prepared. Methylation of 3α,5-cyclo-5α-cholestan-6-one at C-7 was followed by stereoselective reduction with $LiAlH_4$. The key transformation was cycloreversion of the mixture of 7α-methyl-3α,5-cyclo-5α-cholestan-6α-ol and 7β-methyl-3α,5-cyclo-5α-cholestan-6β-ol. The reaction of the latter was much faster under the standard conditions enabling easy separation of the C-7 isomers.

1. Introduction

The C-7 position of the steroid skeleton is a site of some important biotransformations. It is well known[1,2] that 7-dehydrocholesterol (provitamin D_3) and cholic acid (a major component of bovine bile) are formed as a result of cholesterol metabolism. In the latter case, the rate-determining step is the conversion of cholesterol to 7α-hydroxycholesterol by the enzyme 7α-hydroxylase.[3] Incorporation of a C-7 substituent stabilises metabolically the steroid molecule and frequently enhances biological activity of compounds, as in certain steroid enzyme inhibitors. 7-Methyl substituted 4-aza and 6-azacholestane derivatives have been recently described as selective inhibitors of human type 1 5α-reductase.[4,5] They were synthesized from the suitably protected 7-methylcholesterol precursors. Many other 7-substituted steroids have shown interesting antiprogestational,[6] antiestrogen,[7] antiandrogen,[8] aldosterone antagonist[9] and aromatase inhibitory[8] activities. Both epimers of 7-methylcholesterol are available by the methods described in the literature,[10,11] however, most of the methods suffer from a low degree of stereoselectivity, lengthy or inefficient synthetic routes and often require difficult separations of diastereomeric mixtures at some stage of the synthesis. Due to the problems of large-scale preparation of

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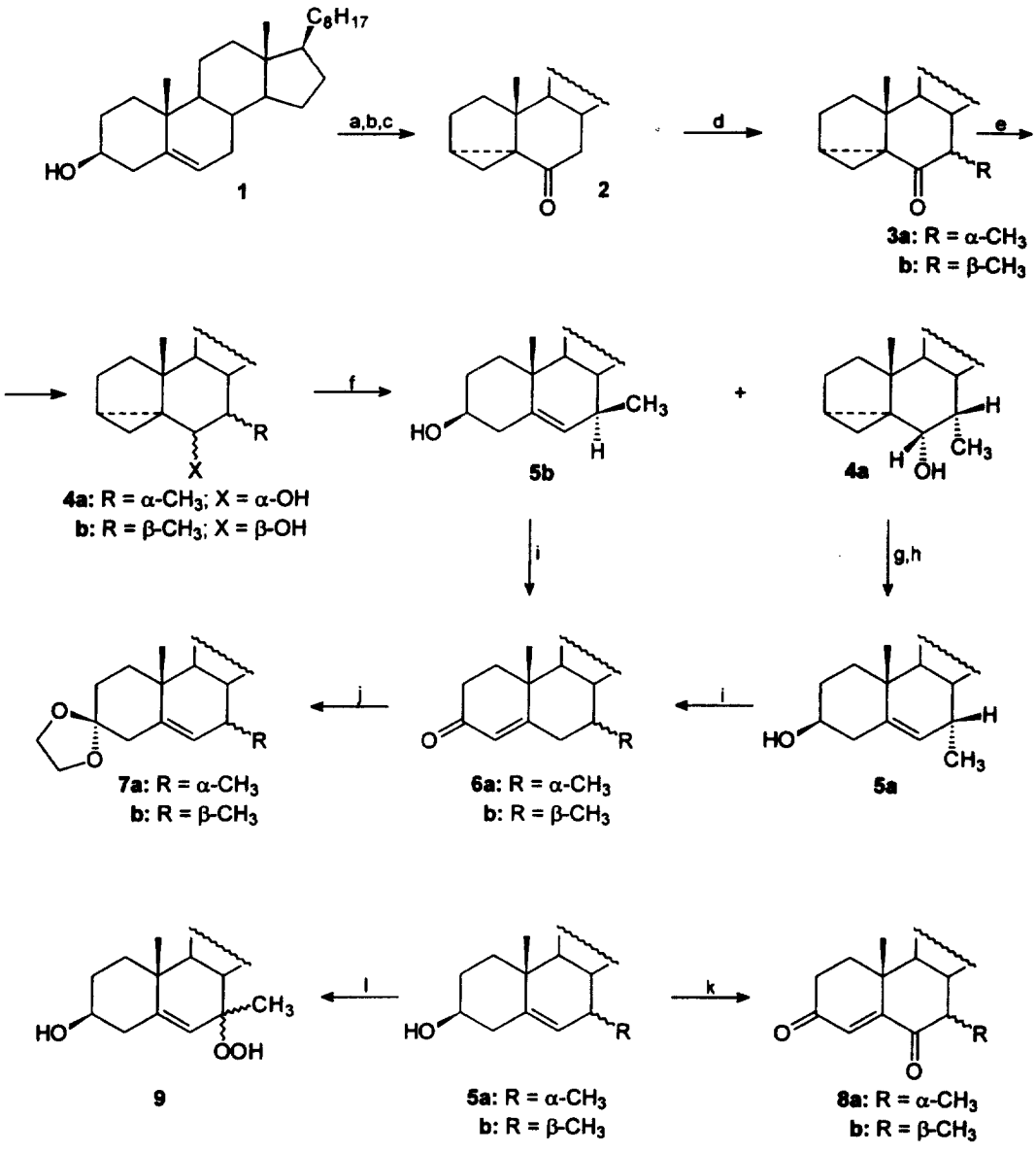
stereochemically pure 7α- or 7β-methylcholesterol, it sometimes proved more convenient[5] to accomplish the synthesis of the desired 7-methylated steroids starting from a C-7 epimeric mixture and then perform careful HPLC separation of final products. There are three major, general approaches to the synthesis of steroids methylated at C-7. In one of them, steroidal dienes, such as 7-methylcholesta-4,6-dien-3-one or 7-methylenecholest-5-en-3β-ol, are regioselectively reduced.[10–12] These dienes, in turn, are available from 7-oxocholesterol via reaction with CH_3MgI followed by the Oppenauer oxidation or via the Peterson olefination. Another approach consists of the conjugate addition of carbanions to steroidal 3-oxo-4,6-dienes.[6,9] In the third method, the 7-methylated steroids are obtained by an allylic bromination of Δ^5-sterols followed by reaction with organometallic compounds.[11,13]

2. Results and discussion

In the present paper, an easy method for concurrent preparation of both C-7 epimers of 7-methylcholesterol **5** and other 7-methylated compounds **6**, **7** and **8** is reported (Scheme 1). The starting material for the synthesis was 3α,5-cyclo-5α-cholestan-6-one **2**,[14,15] readily available from cholesterol. Treatment of **2** with methyl iodide/LDA afforded a 3:2 mixture[16] of 7α- and 7β-methyl derivatives **3a** and **3b**. The mixture of isomers was practically inseparable by chromatography on silica gel. Small amounts of pure C-7 epimers were obtained by semipreparative HPLC and subjected to the $LiAlH_4$ or $LiAlH(O\text{-}t\text{-}Bu)_3$ reduction. Irrespective of the hydride source used, the reduction of the 6-carbonyl group was highly stereoselective. The reactions were stereocontrolled by the configuration of the neighbouring methyl group (hydride approach from the less hindered side). Thus, reduction of 7α-methyl-6-ketone **3a** afforded the corresponding 6α-alcohol **4a**, whereas the epimeric 7β-methyl 6-ketone **3b** gave 6β-alcohol **4b**. The diastereomeric mixture of ketones was reduced with $LiAlH_4$ to the mixture of alcohols **4a** and **4b**, which still appeared to be very difficult to separate. Therefore, the mixture was subjected to aqueous acidic conditions in order to deprotect the $C_{(5)}$–$C_{(6)}$ double bond and the 3β-OH group. According to expectations, the 6β-alcohol **4b** underwent the cycloreversion under the standard conditions (dioxan, water, *p*-TsOH) much faster than **4a**, for stereoelectronic reasons.[17] The separation of the resulting mixture of unreacted **4a** and 7β-methylcholesterol **5b** appeared to be very easy. Their R_f values measured for the silica gel TLC plates developed in benzene:ethyl acetate (9:1) were 0.50 and 0.23, respectively. Flash chromatography yielded both compounds in their pure state and the former was subjected to solvolysis under enforced conditions (acetic acid, diluted sulfuric acid). The alkaline hydrolysis of the crude product afforded 7α-methylcholesterol **5a**. Surprisingly, it was found that 7β-methylcholesterol **5b** had limited stability in solution. It underwent an allylic oxidation with oxygen to give an epimeric mixture of 7*R* and 7*S* hydroperoxides **9** in the ratio 3:2. However, the use of degassed solvents can easily circumvent the autoxidation. The problems with oxygen in 7β-methyl steroid systems were reported earlier.[10] 7α-Methylcholesterol **5a** did not undergo a similar autoxidation, probably because its 7β-proton is less accessible to oxygen.

Oppenauer oxidation of both 7-methylcholesterols **5a** and **5b** afforded the corresponding 7-methylcholest-4-en-3-ones **6a** or **6b** in high yield. Their ketalisation to **7a** or **7b** proceeded smoothly with a simultaneous double bond shift. Oxidation of 7α- or 7β-methylcholesterol with CPA (CrO_3/Py/AcOH) yielded 7-methylcholest-4-en-3,6-diones **8a** or **8b**. All these 7-methylated compounds will be used for the synthesis of steroid aza-analogues.

The chemistry described here for the concurrent synthesis of 7α- and 7β-methylcholesterol should be applicable for the synthesis of other 7-substituted steroids and should help in developing new biologically active steroids.



Scheme 1. Reagents and conditions: (a) *p*-TsCl, py, r.t.; (b) acetone–H_2O, AcOK, b.p.; (c) Sarett reagent; (d) LDA, MeI; (e) $LiAlH_4$, THF, r.t.; (f) dioxan–H_2O, *p*-TsOH, 80°C, 16 h; (g) AcOH, H_2SO_4, r.t., 6 h; (h) KOH, MeOH, b.p.; (i) Oppenauer oxidation; (j) ethylene glycol, *p*-TsOH, Δ; (k) CrO_3, py, AcOH; (l) O_2

3. Experimental

Melting points were determined on a Kofler apparatus of the Boëtius type and were uncorrected. NMR spectra were taken with a Bruker AC 200F spectrometer using $CDCl_3$ solutions with TMS as an internal standard. Infrared spectra were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer for chloroform solutions unless stated otherwise. Mass spectra were obtained at 70 eV with an AMD-604 spectrometer. The reaction products were isolated by column chromatography performed on 70–230 mesh silica gel (J. T. Baker). Thin-layer chromatograms were developed on aluminum TLC sheets

precoated with silica gel F_{254} and visualized with 50% sulfuric acid after heating. All solvents were dried and freshly distilled prior to use. 3α,5-Cyclo-5α-cholestan-6-one **2** was prepared from cholesterol according to the known procedure.[14]

3.1. Methylation of 3α,5-cyclo-5α-cholestan-6-one 2

To the stirred solution of ketone **2** (604 mg; 1.57 mmol) in anhydrous THF (8 mL) was added dropwise 0.79 mL of 2 M LDA solution (Aldrich) at 0°C under argon. After 10 min, methyl iodide (0.12 mL; 1.90 mmol) was added and stirring was continued for 45 min. The reaction was carefully quenched with ethanol (0.5 mL) and poured into water. The product was extracted with benzene and purified by silica gel column chromatography. The 7α- and 7β-methyl ketone mixture of **3a** and **3b** was eluted (545 mg; 87%) with benzene.

Small samples of pure **3a** and **3b** were obtained by semipreparative HPLC separation of the mixture.

Compound **3a**: mp 151–154°C (hexane); IR, ν_{max} 1674, 1298 cm^{-1}; ^1H NMR, δ 2.41 (dq, J=4.7, 7.4 Hz, 1H, 7β-H), 1.06 (d, J=7.4 Hz, 3H, 7α-CH_3), 0.99 (s, 3H, 19-H), 0.71 (s, 3H, 18-H), 0.61 (t, J=4.0 Hz, 1H, cyclopropane-H); ^{13}C NMR, δ 214.6 (C), 56.0 (CH), 51.2 (CH), 47.7 (C), 45.9 (CH), 44.1 (C), 42.6 (C), 39.51 (CH_2), 39.48 (CH_2), 38.2 (CH), 36.7 (CH), 36.1 (CH_2), 35.7 (CH), 35.3 (CH), 33.6 (CH_2), 28.1 (CH_2), 28.0 (CH), 26.1 (CH_2), 23.8 (CH_2), 23.4 (CH_2), 22.8 (CH_2 and CH_3), 22.5 (CH_3), 19.6 (CH_3), 18.7 (CH_3), 13.8 (CH_3), 11.8 (CH_3), 9.8 (CH_2); MS, m/z 398 (M^+, 100), 383 (21), 380 (10), 369 (16); exact mass calcd for $C_{28}H_{46}O$: 398.3549; found: 398.3577.

Compound **3b**: an oil; IR, ν_{max} 1663, 1298 cm^{-1}; ^1H NMR, δ 2.04 (7α-H overlapped with signals of other protons), 1.19 (d, J=7.3 Hz, 3H, 7β-CH_3), 1.03 (s, 3H, 19-H), 0.74 (s, 3H, 18-H); ^{13}C NMR, δ 214.4 (C), 57.7 (CH), 55.7 (CH), 46.3 (C), 46.2 (CH), 45.9 (CH), 43.6 (C), 43.4 (C), 41.1 (CH), 40.1 (CH_2), 39.5 (CH_2), 37.9 (CH), 36.1 (CH_2), 35.8 (CH), 33.5 (CH_2), 28.3 (CH_2), 28.0 (CH), 25.7 (CH_2), 24.9 (CH_2), 23.9 (CH_2), 23.3 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 19.7 (CH_3), 19.6 (CH_3), 18.8 (CH_3), 15.9 (CH_2), 12.4 (CH_3); MS, m/z 398 (M^+, 100), 383 (23), 380 (27), 369 (19); exact mass calcd for $C_{28}H_{46}O$: 398.3549; found: 398.3558.

3.2. Reduction of a mixture of 7α- and 7β-methyl-3α,5-cyclo-5α-cholestan-6-ones 3a and 3b with $LiAlH_4$

The mixture of ketones **3a** and **3b** (530 mg; 1.33 mmol) was dissolved in 10 mL of anhydrous THF and $LiAlH_4$ (260 mg; 6.84 mmol) was added. The reaction mixture was stirred overnight, and then an excess of hydride was carefully decomposed with a few drops of water. The mixture of alcohols **4a** and **4b** was extracted with benzene and subjected to cycloreversion without further purification.

Samples of pure **4a** or **4b** were obtained by reduction of the corresponding epimers of ketone **3**.

Compound **4a**: an oil; IR, ν_{max} 3605, 3417, 1023, 1017 cm^{-1}; ^1H NMR, δ 4.01 (d, J=4.5 Hz, 1H, 6β-H), 0.92 (s, 3H, 19-H), 0.80 (d, J=7.1 Hz, 3H, 7α-CH_3), 0.70 (s, 3H, 18-H), 0.51 (dd, J=8.1, 4.7 Hz, 1H, cyclopropane-H), 0.10 (t, J=4.2 Hz, 1H, cyclopropane-H); ^{13}C NMR, δ 69.6 (CH), 56.2 (CH), 51.9 (CH), 44.9 (C), 42.6 (C), 39.9 (CH_2), 39.5 (CH_2), 39.2 (CH), 38.0 (CH), 37.7 (CH), 36.1 (C and CH_2), 35.7 (CH), 33.0 (CH_2), 28.1 (CH_2), 28.0 (CH), 24.7 (CH_2), 23.8 (CH_2), 23.5 (CH_2), 23.2 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 20.7 (CH), 18.7 (CH_3), 18.0 (CH_3), 11.9 (CH_3), 6.2 (CH_3), 4.9 (CH_2); MS, m/z 400 (M^+, 8), 385 (100), 382 (5), 367 (8), 345 (45); exact mass calcd for $C_{28}H_{48}O$: 400.3705; found: 400.3719.

Compound **4b**: an oil; IR, ν_{max} 3636, 3597, 3444, 1019 cm^{-1}; ^1H NMR, δ 3.46 (d, J=8.8 Hz, 1H, 6α-H), 0.91 (s, 3H, 19-H), 0.90 (d, 3H, 7β-CH_3), 0.70 (s, 3H, 18-H), 0.61 (dd, J=8.1, 4.5 Hz, 1H, cyclopropane-H), 0.24 (t, J=4.0 Hz, 1H, cyclopropane-H); ^{13}C NMR, δ 72.9 (CH), 57.5 (CH), 55.1 (CH),

47.5 (CH), 44.4 (C), 44.2 (C), 43.6 (CH), 42.3 (CH), 40.3 (CH_2), 39.3 (CH_2), 38.7 (C), 36.1 (CH_2), 35.7 (CH), 33.0 (CH_2), 28.6 (CH_2), 28.1 (CH_2), 28.0 (CH), 24.9 (CH_2), 24.0 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 19.1 (CH), 18.9 (CH_3), 18.8 (CH_3), 17.8 (CH_3), 12.3 (CH_3), 7.0 (CH_2); MS, m/z 400 (M^+, 33), 385 (100), 382 (47), 367 (23), 345 (48); exact mass calcd for $C_{28}H_{48}O$: 400.3705; found: 400.3714.

3.3. Selective cycloreversion of alcohol **4b** in the diastereomeric mixture **4a** and **4b**

The mixture of alcohols **4a** and **4b** (516 mg; 1.29 mmol) was dissolved in 10 mL of dioxan:water (9:1), *p*-TsOH (20 mg; 0.11 mmol) was added and the solution was heated at 80°C for 16 h. The reaction mixture was then diluted with water, extracted with chloroform and subjected to silica gel column chromatography. Unchanged 7α-methyl-3α,5-cyclo-5α-cholestan-6α-ol (**4a**; 272 mg) was eluted with benzene. Further elution with benzene:ethyl acetate (9:1) afforded 7β-methylcholesterol **5b** (194 mg).

Compound **5b**: mp 67–70°C; IR, ν_{max} 3604, 3434, 1049, 1034 cm^{-1}; ^1H NMR, δ 5.08 (m, 1H, 6-H), 3.52 (m, 1H, 3α-H), 0.97 (s, 3H, 19-H), 0.96 (d, J=6.8 Hz, 3H, 7β-CH_3), 0.69 (s, 3H, 18-H); ^{13}C NMR, δ 139.2 (C), 129.4 (CH), 71.5 (CH), 57.5 (CH), 55.6 (CH), 51.0 (CH), 43.3 (C), 42.1 (CH_2), 40.1 (CH_2), 39.7 (CH_2), 39.5 (CH_2), 37.2 (CH_2), 36.2 (CH_2), 36.1 (CH), 35.8 (C), 35.7 (CH), 31.8 (CH_2), 28.5 (CH_2), 28.0 (CH), 26.8 (CH_2), 23.9 (CH_2), 22.8 (2×CH_3), 22.5 (CH_3), 21.6 (CH_2), 19.3 (CH_3), 18.8 (CH_3), 12.2 (CH_3); MS, *m/z* 400 (M^+, 24), 385 (25), 382 (100), 367 (14); exact mass calcd for $C_{28}H_{48}O$: 400.3705; found: 400.3695.

3.4. Cycloreversion of 7α-methyl-3α,5-cyclo-5α-cholestan-6α-ol **4a**

Compound **4a** (185 mg; 0.46 mmol) was dissolved in 10 mL of glacial acetic acid, 0.2 mL of 66% H_2SO_4 was added and the reaction mixture was stirred for 6 h at room temperature. The dark reaction mixture was poured into water, neutralized with an NaOH solution and extracted with benzene. The crude product was dissolved in 12 mL of methanol, KOH (400 mg; 7.14 mmol) was added and the solution was heated at reflux for 30 min. The mixture was diluted with water, acidified with aqueous HCl and extracted with chloroform. The product, 7α-methylcholesterol (**5a**; 152 mg) was purified by crystallization from hexane.

Compound **5a**: mp 119–121°C (hexane); lit.[11] mp 128–129°C (petroleum ether); IR, ν_{max} 3604, 3433, 1050, 1040 cm^{-1}; ^1H NMR, δ 5.36 (d, J=5.1 Hz, 1H, 6-H), 3.52 (m, 1H, 3α-H), 1.01 (s, 3H, 19-H), 0.81 (d, J=6.9 Hz, 3H, 7α-CH_3), 0.67 (s, 3H, 18-H); ^{13}C NMR, δ 139.1 (C), 129.1 (CH), 71.9 (CH), 55.9 (CH), 50.0 (CH), 42.4 (CH), 42.3 (CH_2), 42.1 (C), 39.5 (CH_2), 39.4 (CH_2), 37.5 (CH_2), 37.3 (C), 36.2 (CH_2), 35.7 (CH), 34.8 (CH), 31.6 (CH_2), 31.5 (CH), 28.1 (CH_2), 28.0 (CH), 24.5 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 20.8 (CH_2), 19.3 (CH_3), 18.8 (CH_3), 15.4 (CH_3), 11.6 (CH_3); MS, m/z 400 (M^+, 29), 385 (22), 382 (100), 367 (15); exact mass calcd for $C_{28}H_{48}O$: 400.3705; found: 400.3691.

3.5. Oppenauer oxidation of 7α-methylcholesterol **5a**

To a boiling solution of 7α-methylcholesterol **5a** (140 mg; 0.35 mmol) in anhydrous toluene (10 mL) and cyclohexanone (1 mL; 9.7 mmol), a suspension of aluminum isopropoxide (100 mg; 0.49 mmol) in 1.5 mL of toluene was added dropwise. The reaction mixture was refluxed for 1.5 h, then an aqueous saturated solution of sodium tartrate was added and toluene was removed by steam distillation. The residue was diluted with water and extracted with chloroform. The crude product was purified by silica gel column chromatography. Elution with petroleum ether:ethyl acetate (92:8) yielded 113 mg of 7α-methylcholest-4-en-3-one **6a**.

Compound **6a**: an oil; IR, ν_{max} 1656, 1614 cm^{-1}; ^1H NMR, δ 5.72 (d, J=1.5 Hz, 1H, 4-H), 1.18 (s, 3H, 19-H), 0.76 (d, J=7.1 Hz, 3H, 7α-CH_3), 0.71 (s, 3H, 18-H); ^{13}C NMR, δ 199.3 (C), 170.3 (C), 125.6 (CH), 56.0 (CH), 52.1 (CH), 46.1 (CH), 42.4 (C), 40.9 (CH_2), 39.5 (CH_2), 39.4 (CH_2), 38.7 (C), 38.1 (CH), 36.1 (CH_2), 35.9 (CH_2), 35.7 (CH), 34.1 (CH_2), 31.5 (CH), 28.1 (CH_2), 28.0 (CH), 23.8 (CH_2), 23.5 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 21.1 (CH_2), 18.6 (CH_3), 17.8 (CH_3), 12.7 (CH_3), 11.9 (CH_3); MS, m/z 398 (M^+, 100), 383 (26), 356 (43), 275 (82); exact mass calcd for $C_{28}H_{46}O$: 398.3549; found: 398.3579.

In a similar manner the Oppenauer oxidation of 7β-methylcholesterol **5b** to 7β-methylcholest-4-en-3-one **6b** was carried out in 81% yield.

Compound **6b**: an oil; IR, ν_{max} 1656, 1616 cm^{-1}; ^1H NMR, δ 5.70 (s, 1H, 4-H), 1.16 (s, 3H, 19-H), 1.05 (d, J=5.9 Hz, 3H, 7β-CH_3), 0.71 (s, 3H, 18-H); ^{13}C NMR, δ 199.6 (C), 171.4 (C), 122.7 (CH), 56.3 (CH), 55.0 (CH), 53.9 (CH), 43.8 (C), 43.1 (CH_2), 42.9 (CH), 39.6 (CH_2), 39.4 (CH_2), 38.4 (CH), 38.3 (C), 36.1 (CH_2), 35.8 (CH_2), 35.6 (CH), 34.0 (CH_2), 28.5 (CH_2), 28.0 (CH), 27.9 (CH_2), 23.8 (CH_2), 23.3 (CH_3), 22.8 (CH_3), 22.5 (CH_3), 21.4 (CH_2), 18.8 (CH_3), 17.5 (CH_3), 12.2 (CH_3); MS, m/z 398 (M^+, 100), 383 (12), 356 (26), 275 (46); exact mass calcd for $C_{28}H_{46}O$: 398.3549; found: 398.3603.

3.6. Ketalisation of 7α-methylcholest-4-en-3-one 6a

7α-Methylcholest-4-en-3-one **6a** (105 mg; 0.26 mmol) was dissolved in 30 mL of ethylene glycol, *p*-TsOH (40 mg; 0.21 mmol) was added and the solvent was distilled slowly from the reaction mixture at 80°C under reduced pressure (ca. 20 mmHg) for about 3.5 h. The residue was diluted with water and extracted with benzene. Chromatography of the crude product on silica gel with petroleum ether:ethyl acetate (95:5) as eluent gave pure ketal **7a** (80 mg).

Compound **7a**: mp 137–140°C (hexane); IR, ν_{max} 1101, 1090, 1010, cm^{-1}; ^1H NMR, δ 5.33 (dd, J=5.1 Hz, 1.8 Hz, 1H, 6-H), 3.94 (m, 4H, ketal-H), 1.03 (s, 3H, 19-H), 0.87 (d, J=7.1 Hz, 3H, 7α-CH_3), 0.68 (s, 3H, 18-H); ^{13}C NMR, δ 138.4 (C), 129.2 (CH), 109.4 (C), 64.3 (CH_2), 64.1 (CH_2), 55.8 (CH), 50.9 (CH), 42.1 (C), 42.0 (CH), 41.7 (CH_2), 39.5 (CH_2), 39.3 (CH_2), 37.4 (C), 36.4 (CH_2), 36.2 (CH_2), 35.7 (CH), 34.8 (CH), 31.6 (CH), 31.1 (CH_2), 28.1 (CH_2), 28.0 (CH), 24.5 (CH_2), 23.8 (CH_2), 22.8 (2×CH_3), 22.5 (CH_3), 20.7 (CH_2), 18.8 (CH_3), 15.4 (CH_3), 11.6 (CH_3); MS, m/z 442 (M^+, 3), 427 (1), 398 (<1), 99 (100); exact mass calcd for $C_{30}H_{50}O_2$: 442.3811; found: 442.3853.

In a similar manner 7β-methylcholest-4-en-3-one ketal **7b** was obtained in 68% yield.

Compound **7b**: mp 108–110°C (hexane); IR, ν_{max} 1143, 1102, 1010 cm^{-1}; ^1H NMR, δ 5.10 (m, 1H, 6-H), 3.96 (m, 4H, ketal-H), 0.98 (s, 3H, 19-H), 0.95 (d, J=6.8 Hz, 3H, 7β-CH_3), 0.69 (s, 3H, 18-H); ^{13}C NMR, δ 138.6 (C), 130.0 (CH), 109.3 (C), 64.4 (CH_2), 64.2 (CH_2), 57.4 (CH), 55.5 (CH), 50.5 (CH), 43.3 (C), 41.5 (CH_2), 40.1 (CH_2), 39.7 (CH), 39.5 (CH_2), 36.2 (CH_2), 36.1 (CH_2), 36.0 (CH), 35.8 (C and CH), 31.1 (CH_2), 28.5 (CH_2), 28.0 (CH), 26.8 (CH_2), 23.8 (CH_2), 22.8 (2×CH_3), 22.5 (CH_3), 21.5 (CH_2), 18.81 (CH_3), 18.77 (CH_3), 12.1 (CH_3); MS, m/z 442 (M^+, 3), 427 (2), 342 (1), 99 (100); exact mass calcd for $C_{30}H_{50}O_2$: 442.3811; found: 442.3863.

3.7. Oxidation of 7α-methylcholesterol 5a with CPA

A solution of CPA (0.8 M) was prepared by portionwise addition of dry, powdered CrO_3 (2 g) to the stirred mixture of pyridine (3.26 mL) and glacial acetic acid (20 mL). During the addition the reaction temperature was maintained below 20°C.

To a solution of 7α-methylcholesterol **5a** (80 mg; 0.20 mmol) in 0.5 mL of glacial acetic acid, the above 0.8 M CPA solution (1 mL) was added dropwise. The reaction mixture was stirred at room

temperature for 45 min, poured into water and extracted with chloroform. The extract was washed with aqueous sodium bicarbonate, water, aqueous HCl, water, and evaporated *in vacuo*. Chromatography of the crude product on silica gel with petroleum ether:ethyl acetate (95:5) as an eluent gave pure compound **8a** (72 mg).

Compound **8a**: an oil; IR, ν_{max} 1687 cm^{-1}; ^1H NMR, δ 6.05 (s, 1H, 4-H), 1.14 (s, 3H, 19-H), 0.99 (d, J=7.4 Hz, 3H, 7α-CH_3), 0.69 (s, 3H, 18-H); ^{13}C NMR, δ 207.1 (C), 199.0 (C), 161.7 (C), 126.0 (CH), 55.7 (CH), 50.6 (CH), 46.4 (CH), 43.7 (CH), 42.4 (C), 40.5 (C), 39.4 (CH_2), 38.8 (CH_2), 36.3 (CH), 36.0 (CH_2), 35.7 (CH_2), 35.5 (CH), 33.8 (CH_2), 27.91 (CH), 27.86 (CH_2), 23.7 (CH_2), 23.2 (CH_2), 22.7 (CH_3), 22.5 (CH_3), 20.8 (CH_2), 18.6 (CH_3), 17.7 (CH_3), 11.7 (CH_3), 10.9 (CH_3); MS, m/z 412 (M^+, 32), 397 (17), 384 (100), 369 (26); exact mass calcd for $C_{28}H_{44}O_2$: 412.3341; found: 412.3327.

In a similar manner 7β-methylcholest-4-en-3,6-dione **8b** was obtained in 88% yield.

Compound **8b**: mp 130–132°C (hexane); IR, ν_{max} 1681 cm^{-1}; ^1H NMR, δ 6.36 (s, 1H, 4-H), 1.27 (d, J=7.0 Hz, 3H, 7β-CH_3), 1.16 (s, 3H, 19-H), 0.74 (s, 3H, 18-H); ^{13}C NMR, δ 204.7 (C), 199.7 (C), 158.5 (C), 125.9 (CH), 57.3 (CH), 55.4 (CH), 50.0 (CH), 48.1 (CH), 43.5 (C), 40.6 (CH), 39.4 (2×CH_2), 37.7 (C), 36.0 (CH_2), 35.7 (CH), 35.1 (CH_2), 33.9 (CH_2), 28.2 (CH_2), 27.9 (CH), 26.2 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 21.2 (CH_2), 18.7 (CH_3), 17.9 (CH_3), 17.2 (CH_3), 12.2 (CH_3); MS, m/z 412 (M^+, 35), 397 (24), 384 (100), 369 (19), 342 (84); exact mass calcd for $C_{28}H_{44}O_2$: 412.3341; found: 412.3343.

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