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Synthesis of polyhydroxysterols (V): efficient and stereospecific synthesis of 24-methylene-cholest-5-ene-3β,7α-diol and its C-7 epimer

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Abstract

This paper describes the efficient and stereospecific synthesis of cytotoxic dihydroxylated sterols, 24-methylene-cholest-5-ene-3β,7α-diol **1**, and its C-7 epimer, 24-methylene-cholest-5-ene-3β,7β-diol **2**. The crux of the synthesis is that the selective allylic oxidation of 24-methylene-cholesteryl acetate proceeds to 24-methylene-7-keto-cholesteryl acetate without extensive byproduct formation from reaction at the Δ24(28) double bond. This methodology may be useful for the preparation of other oxysterols with non-standard side chains.

Keywords: Sterol; 24-Methylene-cholest-5-ene-3β,7α-diol; 24-Methylene-cholest-5-ene-3β,7β-diol; Synthesis; PCC/$A1_2O_3$

1. Introduction

7-Hydroxy derivatives of oxysterols are of considerable interest because of their biological activities and potential use as intermediates in biosynthesis. 24-Methylene-cholest-5-ene-3β,7α-diol (**1**), first isolated from soft coral *Pseudobersama mossambicensis*, showed significant DNA-damaging activity [1]; 24-Methylene-cholest-5-ene-3β,7β-diol (**2**), first isolated from sponge *Stelodoryx chorophylla* [2], showed moderate cytotoxic activity in the Vero cells assay [3]. **1** and **2** have been previously prepared by an alternative route [3], different from that described herein, requiring isolation from a mixture of 7α- and 7β-isomers. This paper presents the full details of an efficient and stereospecific synthesis of **1** and **2** with greatly improved yields. A key benefit of our method is selective allylic oxidation of 24-methylene-cholesteryl acetate at C7 without extensive byproduct formation from reaction at Δ24(28) double bond.

2. Experimental

2.1. General methods

Melting points were determined on a X_6 melting point apparatus and are uncorrected. Optical rotation was recorded on a Schmidt-Haensch Polaptronic HNQW 5 polarimeter. Infrared spectra were measured in KBr pellets on an EQUINOX 55 FT Spectrophotometer (Bruker Co., Germany). ^1H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA spectrometer at 500 and 125 MHz, respectively, using TMS as an internal standard. Chemical shifts were expressed as ppm (δ) values, and coupling constants (J) were expressed in Hz. MS data were measured on a VG ZAB-HS mass spectrometer. Silica gel 200–300 μm (Qingdao Chemical Industries Co. Ltd., PR China) was used for flash chromatography.

Stigmasterol, methyltriphenylphosphonium bromide, and NaH-paraffin (60% sodium hydride) were purchased from Acros Organics (New Jersey, USA). All chemicals and solvents were of analytical grade. The solvents were purified by general methods, and dimethylsulfoxide (DMSO) was distilled from calcium hydride (CaH_2) before use. PCC/$A1_2O_3$ (pyridinium chlorochromate adsorbed on alumina) was pre-

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pared according to the literature [4] and further dried in vacuo (3 mm Hg) for 2 h at 100 °C. The key intermediate 24-keto-cholesteryl acetate **4** was prepared according to the previously reported method [5] in five steps with an overall 64% yield.

2.2. 24-Keto-cholesterol **5**

24-Keto-cholesteryl acetate **4** (220 mg, 0.5 mmol) was refluxed with 15 ml of 3% KOH in MeOH for 15 min. The solvent was evaporated to a small volume and then diluted with 20 ml ethyl acetate. The organic layer was washed with water and brine. After drying over anhydrous Na_2SO_4, the solvent was removed under reduced pressure, and the resulting crude product was purified by flash chromatography on silica gel using petroleum ether (bp 60–90 °C)/EtOAc (4:1) as eluent and recrystallized from acetone to afford compound **5** (172 mg, 86%). m.p. 128–130 °C. FABMS *m/z*: 401 $(M+H)^+$. IR (KBr) ν: 3416, 2866, 1707, 1640, 1581, 1461, 1377, 1243, 1103, 1053, 1025, 955 cm^{-1}. ^1H NMR ($CDC1_3$, 500 MHz) δ: 0.68 (s, 3H, 18-CH_3), 0.92 (d, 3H, J = 7.0 Hz, 21-CH_3), 1.01 (s, 3H, 19-CH3), 1.09 (d, 6H, two overlapping, J = 7.0 Hz, 26-CH_3 and 27-CH_3), 2.61 (m, 1H, J = 7.0 Hz, 25-CH), 3.53 (tt, 1H, J = 12.0 and 5.5 Hz, 3-CH). 5.35 (dt, 1H, J = 3.0 and 2.5 Hz, 6-CH). Analysis calculated for $C_{27}H_{44}O_2$: C, 81.00; H, 11.00. Found: C, 80.95; H, 11.01.

2.3. 24-Methylene-cholesterol **6**

NaH-paraffin (60%; 84 mg, 2.10 mmol of sodium hydride) was placed in a two-neck flask. After the paraffin was washed away with anhydrous petroleum ether (30–60 °C), 4 ml of anhydrous dimethyl sulfoxide were added to the dried NaH under an argon atmosphere. The mixture was stirred at 80 °C for 40 min, and a dark green solution was produced. After cooling to room temperature, a solution of methyltriphenylphosphonium bromide (500 mg, 1.25 mmol) in 4 ml anhydrous dimethyl sulfoxide was added, and the solution immediately turned yellowish in color. After 10 min, a solution of 24-keto-cholesterol **5** (45 mg, 0.12 mmol) in l ml of anhydrous dioxane was added, and the resulting mixture was stirred at 80 °C for 2 h. The reaction mixture was cooled, diluted with 15 ml water, and extracted with ether (10 ml × 4). The combined organic extracts were washed with water and brine and then, dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the resulting crude product was purified by flash chromatography on silica gel using petroleum ether (bp 60–90 °C)/EtOAc (5:1) as eluent and recrystallized from methanol to afford colorless crystal needles of **6** (39 mg, 85%). m.p. 140–142 °C (literature value: 143 °C [6]); $[\alpha]_D = -36°$ (*c*, 0.15, $CHCl_3$) (literature value: −34.8° (*c*, 0.26, $CHCl_3$) [6]). FABMS *m/z*: 399 $(M+H)^+$. 1R (KBr) ν: 3416, 3078, 2942, 1644, 1461, 1377, 1053, 955, 885, 842, 800 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.68 (s, 3H, 18-CH_3), 0.95 (d, 3H, J = 6.5Hz, 21-CH_3), 1.01 (s, 3H, 19-CH_3), 1.02(d, 3H, J = 7.0 Hz, 26-CH_3 or 27-CH_3), 1.03 (d, 3H, J = 7.0 Hz, 26-CH_3 or 27-CH_3), 3.54 (tt, 1H, J = 11.5 and 5.0 Hz, 3-CH), 4.66 (d, 1H, J = 1.5 Hz, 28-CHa), 4.71 (d, 1H, J = 1.5 Hz, 28-CHb), 5.35 (dt, 1H, J = 3.0 and 2.5 Hz, 6-CH); ^{13}C NMR ($CDC1_3$, 125 MHz) δ: 156.9 (C), 140.7 (C), 121.7 (CH), 105.9 (CH_2), 71.9 (CH), 56.8 (CH), 56.0 (CH), 50.1 (CH), 42.3 (CH_2), 42.3 (C), 39.8 (CH_2), 37.2 (CH_2), 36.5 (C), 35.7 (CH), 34.7 (CH_2), 33.8 (CH), 31.9 (CH_2), 31.9 (CH), 31.6 (CH_2), 30.9 (CH_2), 28.2 (CH_2), 24.3 (CH_2), 22.0 (CH_3), 21.8 (CH_3), 21.1 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3). Analysis calculated for $C_{28}H_{46}O$: C, 84.42; H, 11.56. Found: C, 84.42; H, 11.57.

2.4. 24-Methylene-7-keto-cholesteryl acetate **7** and 7,24-Diketo-cholesteryl acetate **8**

24-Methylene-cholesterol **6** (50 mg, 0.13 mmol) was dissolved in 2 ml pyridine, and l ml acetic anhydride was added. The mixture was incubated at room temperature for 24 h. Water (10 ml) was added to the mixture to quench the excess acetic anhydride, and the mixture was extracted with ethyl acetate (10 ml × 4). The combined organic layer was washed successively with water, 1 M HC1 solution, and brine, and then dried over anhydrous Na_2SO_4. The solvent was removed and the dried residue was dissolved in 15 ml benzene, containing 50 mg molecular sieves (3A). PCC/Al_2O_3 (2.0 g) was added, and the mixture was stirred with in reflux for 24 h under nitrogen. After cooling to room temperature, the mixture was thoroughly extracted with CH_2Cl_2. After evaporation to dryness under reduced pressure, the residue was subjected to flash chromatography on silica gel using petroleum ether (bp 60–90 °C)/EtOAc (7:1) as eluent, and the acetate of substrate **6** (R_f = 0.75) 11 mg was recovered. The followed portion (R_f = 0.30) was collected and recrystallized from ether/methanol to afford **7** (29 mg, 51%). m.p. 154–155 °C. FABMS *m/z*: 455 $(M+H)^+$. IR (KBr) ν: 3078, 2959, 2872, 1731, 1673, 1639, 1465, 1376, 1263, 1239, 1185, 1044, 887 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.69 (s, 3H, 18-CH_3), 0.96 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.02 (d, 3H, J = 7.0 Hz, 26-CH_3 or 27-CH_3), 1.03 (d, 3H, J = 7.0 Hz, 26-CH_3 or 27-CH_3), 1.21 (s 3H, 19-CH_3), 2.06 (s, 3H, 3-CH_3COO), 4.66 (d, 1H, J = 1.5 Hz, 28-CHa), 4.72 (d, 1H, J = 1.5 Hz, 28-CHb), 4.72 (m, 1H, 3-CH), 5.71 (d, 1H, J = 1.5 Hz, 6-CH). Analysis calculated for $C_{30}H_{46}O_3$: C, 79.30; H, 10.13. Found: C, 79.23; H, 10.18. The portion (R_f = 0.12) was collected using petroleum ether (bp 60–90 °C)/EtOAc (4:1) as eluent and recrystallized from ether/methanol to afford **8** (9 mg, 16%). m.p. 141–143 °C. FABMS *m/z*: 457 $(M+H)^+$. IR (KBr) ν: 2949, 2872, 1733, 1711, 1671, 1631, 1465, 1379, 1243, 1183, 1038 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ: 0.68 (s, 3H, 18-CH_3), 0.92 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.09 (d, 6H, two overlapping, J = 7.0 Hz, 26-CH_3 and 27-CH_3), 1.21 (s, 3H, 19-CH_3), 2.06 (s, 3H, 3-CH_3COO), 2.61 (m, 1H, J = 7.0 Hz, 25-CH), 4.72 (m, 1H, 3-CH), 5.71 (d, 1H, J = 2.0 Hz, 6-CH). Analysis calculated for $C_{29}H_{44}O_4$: C, 76.32; H, 9.65. Found: C, 76.31; H, 9.70.

2.5. 24-Methylene-cholest-5-ene-3β, 7α-diol **1**

24-Methylene-7-keto-cholesteryl acetate **7** (35 mg, 0.077 mmol) was dissolved in 3 ml dry THF at −78 °C, and 0.6 ml of 1 M L-selectride in THF was added. The resulting solution was stirred at −78 °C for 30 min under nitrogen, and l ml acetone was added. The solvent was evaporated, and the residue was treated with 5 ml of 5% HCl and 10 ml ethyl acetate. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and then, evaporated to give a white solid, which was dissolved in 5 ml THF and 2 ml methanol, containing 3% potassium hydroxide. The mixture was then treated in a similar way as described in 2.2. The crude product was purified by flash chromatography on silica gel using petroleum ether (bp 60–90 °C)/EtOAc (2:1) as eluent and recrystallized from methanol to afford compound **1** (19 mg, 60%). m.p. 191–193 °C (literature value: 193–195 °C [1]); $[\alpha]_D = -79°$ (*c*, 0.21, $CHCl_3$) (literature value: −85° (*c*, 0.27, $CHCl_3$) [1]). FABMS *m/z*: 415 $(M+H)^+$. IR (KBr) *ν*: 3322, 2935, 2867, 1635, 1540, 1465, 1380, 1195, 1056, 1009, 953, 808 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) *δ*: 0.69 (s, 3H, 18-CH_3), 0.96 (d, 3H, *J* = 6.5 Hz, 21-CH_3), 1.00 (s, 3H, 19-CH_3), 1.02 (d, 3H, *J* = 7.0 Hz, 26-CH_3 or 27-CH_3), 1.03 (d, 3H, *J* = 7.0 Hz, 26-CH_3 or 27-CH_3), 3.59 (m, 1H, 3-CH), 3.85 (brs, 1H, 7-CH), 4.66 (brs, 1H, 28-CHa), 4.72 (brs, 1H, 28-CHb), 5.61 (dd, 1H, *J* = 5.5 and 2.0 Hz, 6-CH). ^{13}CNMR ($CDCl_3$, 125 MHz) *δ*: 11.6 (CH_3), 18.2 (CH_3), 18.7 (CH_3), 20.7 (CH_2), 21.8 (CH_3), 21.9 (CH_3), 24.2 (CH_2), 28.2 (CH_2), 30.8 (CH_2), 31.3 (CH_2), 33.7 (CH), 34.6 (CH_2), 35.6 (CH), 37.0 (CH_2), 37.4 (C), 37.5 (CH), 39.1 (CH_2), 42.0 (CH_2), 42.1 (C), 42.2 (CH), 49.4 (CH), 55.6 (CH), 65.3 (CH), 71.3 (CH), 105.9 (CH_2), 123.8 (CH), 146.2 (C), 156.9 (C). Analysis calculated for $C_{28}H_{46}O_2$: C, 81.16; H, 11.11. Found: C, 81.14; H, 11.15.

2.6. 24-Methylene-cholest-5-ene-3β, 7β-diol **2**

24-Methylene-7-keto-cholesteryl acetate **7** (30 mg, 0.066 mmol) and $CeCl_3{\cdot}7H_2O$ (25 mg, 0.067 mmol) were dissolved in 3 ml THF/MeOH (2:1). Sodium borohydride (10 mg, 0.26 mmol) was added slowly while stirring. After 5 min, the reaction was quenched with 5% HCl (l ml), and the solution was extracted with ethyl acetate (10 ml × 3) and dried over anhydrous Na_2SO_4. The solvent was removed and the residue was dissolved in 5 ml THF and 2 ml methanol, containing 3% potassium hydroxide. The mixture was then treated in a similar way as described in 2.2. The crude product was purified by flash chromatography on silica gel using petroleum ether (bp 60–90 °C)/EtOAc (2:1) as eluent and recrystallized from methanol/water to afford compound **2** (18 mg, 65%). m.p. 150–152 °C (literature value: 152–153 °C [3]); $[\alpha]_D = +22°$ (*c*, 0.13, $CHCl_3$) (literature value: +19° (*c*, 0.16, $CHC1_3$) [3]). FABMS *m/z*: 415 $(M+H)^+$. IR (KBr) *ν*: 3338, 2935, 2902, 2869, 1650, 1465, 1379, 1303, 1056, 1014, 951 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) *δ*: 0.70 (s, 3H, 18-CH_3), 0.96 (d, 3H, *J* = 6.5 Hz, 21-CH_3), 1.02 (d, 3H, *J* = 7.0 Hz, 26-CH_3 or 27-CH_3), 1.03 (d, 3H, *J* = 7.0 Hz, 26-CH_3 or 27-CH_3), 1.05 (s, 3H, 19-CH_3), 3.55 (m, 1H, 3-CH), 3.85 (dt, 1H, *J* = 8.0, 2.0 Hz, 7-CH), 4.66 (brs, 1H, 28-CHa), 4.72 (brs, 1H, 28-CHb), 5.29 (t, 1H, *J* = 3.0 Hz, 6-CH). ^{13}CNMR ($CDCl_3$, 125 MHz) *δ*: 11.8 (CH_3), 18.7 (CH_3), 19.1 (CH_3), 21.0 (CH_2), 21.8 (CH_3), 21.9 (CH_3), 26.3 (CH_2), 28.5 (CH_2), 31.0 (CH_2), 31.5 (CH_2), 33.8 (CH), 34.7 (CH_2), 35.6 (CH), 36.4 (C), 36.9 (CH_2), 39.5 (CH_2), 40.9 (CH), 41.7 (CH_2), 43.0 (C), 48.2 (CH), 55.3 (CH), 55.9 (CH), 71.4 (CH), 73.3 (CH), 105.9 (CH_2), 125.4 (CH), 143.5 (C), 156.9 (C). Analysis calculated for $C_{28}H_{46}O_2$: C, 81.16; H, 11.11. Found: C, 81.10; H, 11.17.

3. Results and discussion

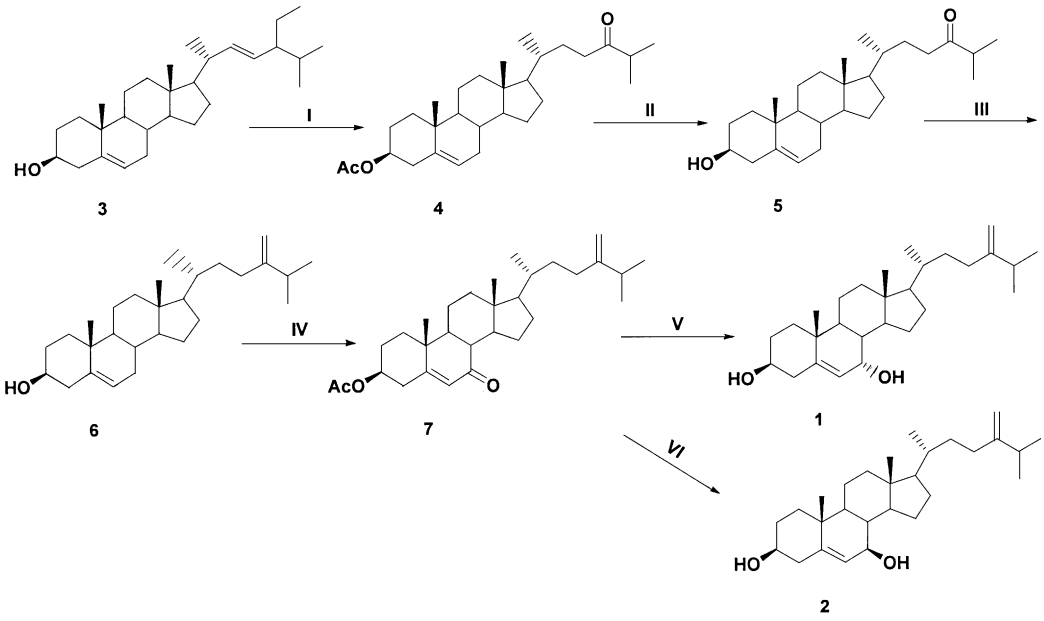
The strategy for the stereospecific synthesis of the dihydroxysterols **1** and **2** is illustrated in Scheme 1. Basically, it involved methylenation of the 24-oxo group, introduction of a 7-oxo group by selective oxidation at C7, and conversion of this 7-oxo group to a 7α-hydroxy or 7β-hydroxy group. **1** and **2** were obtained from **4** in 22.4 and 24.2% yield, respectively.

In the literature [3], allylic hydroxylation of **4** has been carried out in AcOH using *tert*-butylperoxybenzoate in the presence of cuprous bromide to yield a mixture of C-7 acetates. The mixture of diacetates was then subjected to hydrolysis using 5% KOH in MeOH to give the 24-keto-cholest-5-ene-3β,7α-diols **9** and 24-keto-cholest-5-ene-3β,7β-diols **10** in a relatively low yield. **9** and **10**, practically not separable by general flash chromatography, were transformed to **1** and **2** by methylenation of 24-oxo group. **1** and **2** were obtained from **4** in 12.5 and 5.9% yield, respectively.

The literature [3] reports that although the above-described method to introduce 7-hydroxy group is non-stereospecific and results in epimeric products, it saved steps. It is also argued that the stereospecific introduction of the 7-hydroxy group from **4** should involve the protection of the 24-carbonyl group as a ketal, followed by allylic oxidation at C-7, stereoselective reduction of the resulting 7-carbonyl group to a 7α-hydroxy or 7β-hydroxy group, and then, removal of the ketal.

In this paper, we pursued an alternative route of stereospecific synthesis of **1** and **2** without the protection and deprotection of the C-24 carbonyl group (Scheme 1). The crux of this synthetic route is the selective allylic oxidation at C7 of the 5,24(28)-diene steroid (the acetate of **6**). This approach is the most feasible since the products have the correct stereochemistry and more reasonable yields are obtained.

Our synthetic design took into account the fact that the readily available steroid stigmasterol **3** has been converted in five steps to the steroid 4 with the correct side-chain structure and absolute stereochemistry [5]. Compound **4** was subjected to hydrolysis using 3% KOH in MeOH to yield the steroid **5**, which was treated with triphenyl phosphonium methylide in



I). Ref.[4]; II). CH_3OH/KOH; III). $Ph_3P=CH_2$; IV). a. Ac_2O/Py, b. PCC/Al_2O_3; V). a. L-selectride, b. CH_3OH/KOH; VI). a. $NaBH_4/CeCl_3{\cdot}7H_2O$, b. CH_3OH/KOH

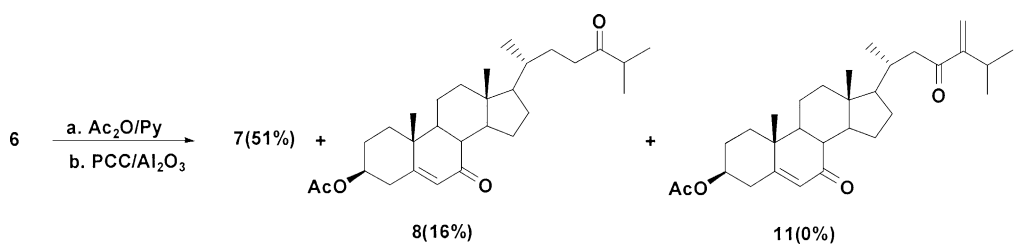
Scheme 1. (I) [4], (II) CH_3OH/KOH, (III) $Ph_3P=CH_2$, (IV) (a) Ac_2O/Py and (b) PCC/Al_2O_3, (V) (a) L-selectride and (b) CH_3OH/KOH, (VI) (a) $NaBH_4/CeCl_3{\cdot}7H_2O$ and (b) CH_3OH/KOH.

DMSO (formed in situ using Ph_3PCH_3Br and NaH in DMSO) to give steroid **6** [7]. After protection of the 3β-OH group with Ac_2O/pyridine, allylic oxidation [8] of **6** was carried out using PCC/Al_2O_3 in the benzene to yield steroid **7** and the unexpected byproduct **8**, without extensive byproduct formation from reaction at the Δ24(28) double bond, i.e., 24-methylene-7,23-diketo-cholesteryl acetate **11** (Scheme 2).

The amount of $PCC/A1_2O_3$ used had a key effect on this reaction; too much $PCC/A1_2O_3$ was favorable for the formation of **8**. Relatively reasonable ratio of oxidant/substrate and reaction time were obtained after extensive investigation (Table 1).

We also found that the allylic oxidation of Δ^5-steroids with PCC/Al_2O_3 prepared according to literature [4] method was only of limited success (most of the substrate was recovered). Its oxidation capacity could be greatly augmented if further dried in vacuo (3 mmHg) for 2 h at 100 °C. The reagent with sufficient dryness was stable in the dark and dried conditions, and it could be kept for months without losing activity compared to several weeks in literature [4]. $PCC/A1_2O_3$ has been first applied in allylic oxidation of Δ^5-steroids [8], and it turned out to be effective, moreover, oxidation with PCC/Al_2O_3 was more profitable than PCC in the working up, which was reduced to a mere filtration through a short silica gel column to get rid of solid impurity.

The physical and spectral data of **6** was in good agreement with the literature [9]. The IR spectrum of **7** showed medium CH stretch at 3078 cm^{-1} and C=C stretch at 1639 cm^{-1}, corresponding to the 24(28) terminal double bond; α,β-unsaturated ketone stretch at 1673 cm^{-1} corresponding to **7**-carbonyl group. The ^1H NMR spectrum of **7** showed the same two broad singlets at δ 4.66 and 4.72 for the 28-H_2 protons as seen for compound **6**. The only observable differences between **6** and **7** in ^1H NMR spectrum were for the 6-H and 19-H_3 protons. In compound **6**, the 6-H proton resonated at δ 5.35 and appeared as a doublet of triplet (J = 3.0 and 2.5 Hz), and the 19-H_3 protons resonated at δ 1.01 as a singlet; in compound **7**, however, the 6-H proton resonated at δ 5.71 and appeared as a doublet (J = 1.5 Hz) due to the substitution



Scheme 2.

Table 1
Oxidation of 24-methylene-cholesteryl acetate withPCC/Al_2O_3[a]

Ratio of oxidant/substrate (g/g)	Time (h)	Recovered (R_f = 0.75[b]; %)	Product **7** (R_f = 0.30[b]; %)	Byproduct **8** (R_f = 0.12[b]; %)	Loss (%)[c]
10	24	63	17	7	13
20	24	41	34	11	14
30	24	32	40	10	18
40	6	53	25	7	15
40	12	25	47	12	16
40	24	20	51	16	13
40	36	19	47	20	14
50	24	16	45	22	17
60	24	17	43	25	15

[a] The reaction was carried out in refluxing benzene, containing 3A molecular sieves under N_2.
[b] The eluent is petroleum ether (bp 60–90 °C)/EtOAc (7:1)
[c] The yield was calculated after separation by flash column.

of 7-H, and the 19-H_3 protons signal was shifted downfield to δ 1.21. The IR spectrum of **8** showed a saturated ketone stretch at 1711 cm^{-1} assigned to the 24-carbonyl group, and the absence of the characteristic absorption of the terminal double bond. The ^1H NMR spectrum of **8** showed no signals of the 28-H_2 protons, and a 1H multiplet at δ 2.61 (J = 7.0 Hz) was assigned to the 25-H, 26-H_3 and 27-H_3 appeared as two overlapped doublets. In compound **7**, however, the 25-H signal was submerged upfield; the 26-H_3 and 27-H_3 appeared as two separated doublets. Shieldings and coupling constants of **7** and **8** in rings A and B were almost identical to those analogs described in literature [10]. The comparison of selective ^1H NMR spectral data for some 24-keto or 24-methylene steroids is shown in Table 2, which confirmed the side-chain structures of **7** and **8**.

Compound **7** was treated with L-selectride in dried THF [10] to yield 7α-hydroxylate, which was subjected to hydrolysis of its 3β-acetoxy group using 3%KOH in MeOH to give the 24-methylene-cholest-5-ene-3β,7α-diol **1**. No evidence of the 7β-epimer could be detected by NMR analysis. Compound **7** was treated with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ in THF/MeOH [10] to yield 7β-hydroxylate, which was subjected to hydrolysis to give the 24-methylene-cholest-5-ene-3β,7β-diol **2**. No evidence of the 7α-epimer could be detected by NMR analysis.

On the basis of their NMR data and literature survey [10–12], the stereo-structures of **1** and **2** were identified. The physical and spectral data of **1** and **2** were in good agreement with the literatures [1,2]. NMR chemical shifts of **1** and **2** matched literature values to ±0.1 ppm for ^{13}C and ±0.01 ppm for ^1H.

Table 2
Selected ^1H NMR spectral data for steroids **5**–**8**, **12**[a] and **1**

Steroid	H-25	H_3-26	H_3-27
5	2.61 (m, J = 7.0 Hz)	1.09	1.09
6	Submerged upfield	1.02	1.03
7	Submerged upfield	1.02	1.03
8	2.61 (m, J = 7.0 Hz)	1.09	1.09
12[a] [1]	2.60 (sept. J = 6.9 Hz)	1.08	1.08
1 [1]	Submerged upfield	1.02	1.03

[a] 24-Keto-cholest-5-en-3β,7α-diol.

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