METHYL STEROIDS. STUDIES ON THE SYNTHESIS OF 4-METHYL- AND 4,4-DIMETHYL-25-HYDROXYCHOLESTAN-3-ONE DERIVATIVES

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The synthetic routes to 25-hydroxy derivatives of 4-methylcholest-4-ene and 4,4-dimethylcholest-5-ene from methyl lithocholate and methyl 3β-acetoxy-24-homochol-5-en-25-oate (**6**) have been investigated. The cholest-5-ene-3β,25-diol (**7**), readily available from **6**, was transformed in a few steps into the title compounds. It was also found that bromination of 24-acetoxy-5β-cholan-3-one (**1**) and of its enol acetate followed by dehydrobromination is not a regioselective reaction. Formation of mixtures of 2β-bromo-3-oxo and 4β-bromo-3-oxo compounds, which gave mixtures of 24-acetoxychol-4-en-3-one (**4**) and 24-acetoxy-5β-chol-1-en-3-one (**5**) of similar polarity was observed. 4-Methyl-25-hydroxycholest-4-en-3-one (**14**) and 4-methyl-25-hydroxycholesta-1,4-dien-3-one (**16**) are potential substrates for the preparation of 4-methyl analogs of vitamin D_3.

Keywords: 25-Hydroxy steroids; 4-Methyl steroids; Alkylation; Methylation; Bromination; Cholestane.

Introduction of methyl groups at various positions in steroids has been the subject of several investigations. It is well known that the biological activity of modified steroids having an additional alkyl group may be enhanced or modified[1]. For example some C-2 methylated 3-ketones unsaturated in position 4 have higher activity than the parent ketones[2]. Introduction of a methyl group at carbon atom C-7 in steroids resulted in compounds showing significant biological activities, especially in the case of 7α-methyl isomers[3]. 4α-Methyl-5α-pregn-1-ene-3,20-dione and 6α-methylpregn-4-ene-3,20-dione derivatives have also been synthesized for testing better hormonal activity[4]. 4,4-Dimethyl derivatives of androstane and pregnane have been reported as well[5].

Steroids with an additional methyl group in ring A are also encountered in natural sources like sediments of different origin[6], marine organisms[7] and pollen grains of *Ambrosia elatior*[8]. A mixture of 4α-methylsterols has been isolated from leaves of potato plants *Solanum tuberosum* L.[9]

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19-Norvitamin D_3 analogs possessing a hydrophobic substituent, e.g. methyl or ethyl in the C-2 position, have been shown to exhibit increased biological activity[10]. In a search for compounds with a better therapeutic index, the synthesis of 4,4-dimethyl-1α-hydroxy-epivitamin D_3 [11], 6-methyl-vitamin D_3 [12] and 4,4-dimethylcalciferol[13] has been published. It has been proposed by Choliński and Kutner that the introduction of an additional substituent into ring A of vitamin D_3, for example the 4-methyl group, can possibly change the conformation of that ring resulting in a modification of interaction of the hormone with the receptor[14]. Thus, the aim of our studies was to synthesize 25-hydroxycholestane derivatives alkylated in position 4.

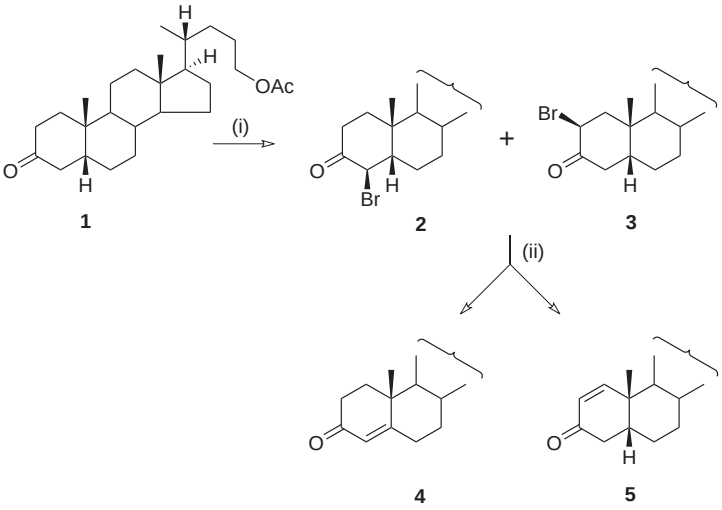
Introduction of the methyl group into ring A of steroids have been reported[6,15]. The most frequently used method was the base-catalyzed alkylation of 3-oxosteroids[4,5,13,16] unsaturated in position 4. While 3-oxo-5α-steroids are methylated in position 2 [16a] or 4 [15b,16b], the 3-oxo-5β counterparts react under alkylating conditions to afford mixtures of 2- and 4-monomethyl as well as 2,4- and 4,4-dimethyl-3-oxo compounds[17,18]. In our planned synthesis of 4-methyl-25-hydroxycholestane derivatives, the readily available lithocholic acid and 24-homochol-5-en-25-oic acid were considered as substrates[19]. Thus, functionalization of ring A and elongation of the side chain of these substrates was required.

It has been shown that bromination of methyl 3-oxo-5β-cholan-25-oate with iodine monobromide proceeds with the formation of 4β- and 2β-bromo-3-oxo derivatives[20] and the extensive migration of bromine from position C-4 to C-2 has been observed during a prolonged reaction time. On the other hand, good yield of 4-bromo derivatives was obtained when various 3-oxo-5β-steroids were brominated in acetic acid or when 3-hydroxy-5β-steroids were treated with *N*-bromosuccinimide and hydrogen bromide[21]. Therefore, we expected selective bromination of 3-oxo-5β-cholane derivatives.

RESULTS AND DISCUSSION

In preliminary experiments oxidation of methyl lithocholate to the respective 3-ketone followed by its bromination–dehydrobromination gave unsatisfactory results due to formation of mixtures of products. Therefore, we considered the 3α,24-dihydroxy-5β-cholane derivative as substrate. The ketone **1** [22] was treated with perchloric acid in acetic anhydride to give enol acetate. Bromination of the crude enol acetate resulted in formation of the mixture of bromo ketones **2** and **3**, which could not be effectively sepa-

rated. This was dehydrobrominated with lithium chloride–lithium carbonate in dimethylformamide to a mixture of enones **4**[23] and **5**. Pure enones were obtained after crystallization and chromatography. The enone **5** was characterized by ^{1}H and ^{13}C NMR, IR and mass spectra (see Experimental). Direct bromination[21] of ketone **1** (Scheme 1) also afforded a mixture of 4β- and 2β-bromo ketones **2** and **3**, which were dehydrobrominated as above to give **4** and **5** in the ratio 5.6:1, assigned from the ^{1}H NMR spectrum. It was therefore concluded that the formation of mixtures of 2- and 4-bromo 3-ketones and of Δ^1- and Δ^4-3-ketones could not be avoided.



(i) 1. Ac_2O, AcOEt, 72% $HClO_4$ cat., 5 h, 2. AcONa, Br_2, AcOH, 1 h;
(ii) LiCl, DMF, Li_2CO_3, reflux, 1 h

SCHEME 1

Consequently, neither methyl 3-oxo-5β-cholan-25-oate nor 24-acetoxy-5β-cholan-3-one (**1**) were not further considered as substrates for efficient functionalization of ring A. Therefore, we turned our attention to homocholanoic acid derivative[19] **6**. The elongation of the side chain of the ester **6** accompanied by the introduction of the 25-hydroxy group was straightforward and the double bond in position 5 could easily be moved into ring A of the steroid. 25-Hydroxycholesterol (**7**) which could be synthesized[24] from ester **6** in two steps in 90% yield (Fig. 1), was considered as an appropriate intermediate in the synthesis of 25-hydroxycholestane derivatives methylated in ring A. Compound **7** has been previously reported as a key

intermediate in the synthesis of a biologically active form of vitamin D_3 [25] and its analogs[26]. In these syntheses the introduction of the 1α-hydroxyl group was achieved via the oxidation–reduction of 1-en-3-oxo[19,26b,27] as well as 1,4,6-trien-3-oxocholestane derivatives[28].

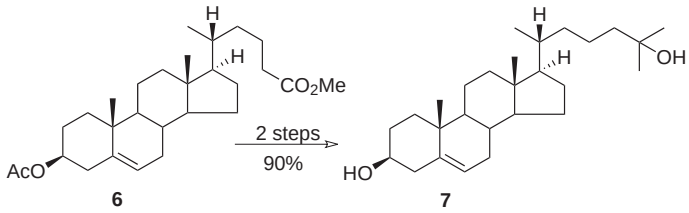
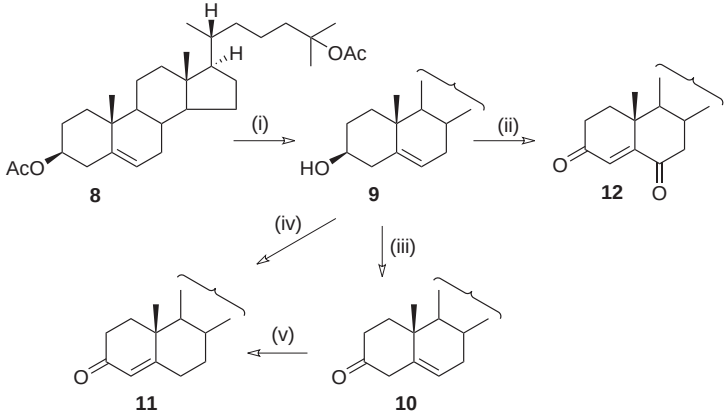


FIG. 1
Synthesis of 25-hydroxycholesterol (**7**)

3β,25-Diacetoxycholest-5-ene[29] (**8**) was hydrolyzed quantitatively to monoacetyl derivative **9** upon mild hydrolysis with potassium carbonate in methanol (Scheme 2).



(i) K_2CO_3, MeOH; (ii) PCC, $CaCO_3$, CH_2Cl_2, 5 h; (iii) PCC, $CaCO_3$, CH_2Cl_2, 1 h; (iv) Al(O*i*-Pr)$_3$, toluene-cyclohexanone, reflux, 2 h; (v) oxalic acid, EtOH, reflux, 1 h

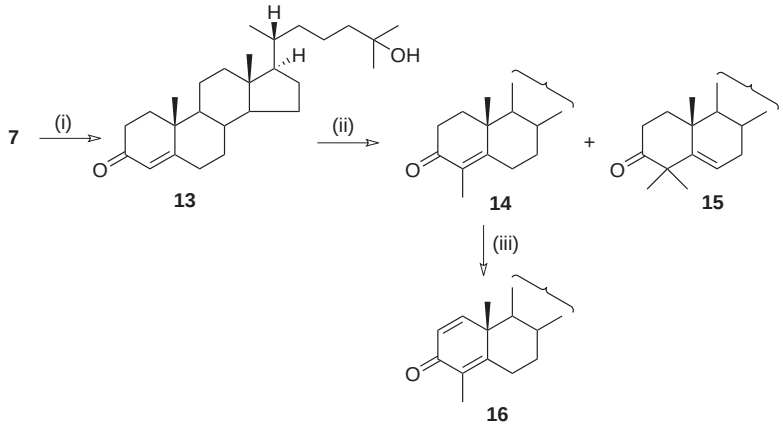
SCHEME 2

The ^{1}H NMR spectrum of **9**, which showed characteristic signals at δ 1.97 and 3.52, confirmed the selective hydrolysis of the 3-acetate group in **8**. The alcohol **9** was oxidized with pyridinium chlorochromate in methylene chloride to afford the unconjugated ketone **10** in 72% yield. The same oxi-

dation carried out in the presence of anhydrous calcium carbonate allowed isolation of compound **10** in 77% yield. In the prolonged time reaction (3 h) formation of enone **11** as a major product or 4-ene-3,6-dione **12** (5 h) has been observed. Compounds **10** and **12** were characterized by ^{1}H and ^{13}C NMR, IR and mass spectra. ^{1}H NMR spectra of compounds **10** and **12** were compared with those of the respective cholestane derivatives[30] and showed similarities with them as expected.

The oxalic acid catalyzed isomerization of enone **10** resulted in the formation of conjugated enone **11** in 91% yield of the crude product; however, pure enone **11** was isolated upon chromatography in 47% yield. The enone **11** was also prepared from **9** in one step under Oppenauer conditions in 64% yield. Compound **11** showed all characteristic spectroscopic properties (IR, ^{1}H and ^{13}C NMR, MS; see Experimental).

The direct oxidation of the diol **7** under Oppenauer conditions offered alternative route to 25-hydroxycholest-4-en-3-one (**13**), which was prepared in 86% yield (Scheme 3). Methylation of the enone **13** with methyl iodide and potassium *tert*-butoxide in *tert*-butanol gave a mixture of mono and



(i) Al(O*i* -Pr)$_3$, toluene-cyclohexanone, reflux, 40 min; (ii) *t*-BuOK, *t*-BuOH, MeI, reflux, 30 min; (iii) dioxane, DDQ, reflux, 22 h

SCHEME 3

dimethyl derivatives **14** and **15** of similar polarity. Compounds **14** and **15** were isolated in 34 and 25% yield, respectively, after chromatography on a silica gel column. Methylation of enone **11** under similar conditions resulted in formation of mono and dimethyl derivatives **14** and **15** in approximately 3:4 ratio along with small amount (less than 5%) of the substrate, as estimated from ^{1}H NMR analysis. The attempts to improve the yield of

the monoalkylated derivative **14** by changing the reaction time and the ratio of reagents were unsuccessful since shorter reaction time resulted in higher recovery of the substrate ketone **11**.

In the final step, dehydrogenation of 4-methyl derivative **14** with excess 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in anhydrous dioxane (100 °C, 22 h) gave the expected dienone **16** in 62% yield after chromatography, along with the substrate which was recovered in 18% yield. 4-Methyl-1,4-dien-3-one **16** showed characteristic signals of 1-H, 2-H and 4-CH_3 protons in the ^{1}H NMR spectrum. Dehydrogenation of enone **15** under similar conditions with various excesses of DDQ gave negative results.

All the new compounds were characterized by ^{1}H NMR, IR and mass spectra. The full assignment of signals in the ^{13}C NMR spectra of 25-hydroxycholestane derivatives is presented in Table I.

In summary, we have developed a synthetic route to 4-methyl and 4,4-dimethyl derivatives of 25-hydroxycholestan-3-one **14**, **15** and **16**. The 25-hydroxycholest-4-en-3-one (**13**), which served as the key intermediate in the synthesis, was prepared from methyl 3β-acetoxy-24-homochol-5-en-25-oate (**6**) in three steps in 78% yield. In the alternative approach to compounds **14** and **15**, synthetically useful new 25-hydroxycholestane derivatives **9**–**12** were obtained, while the intermediate 25-acetoxycholest-4-en-3-one (**11**) was prepared from the 3,25-diacetate **8** in 63% yield.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were determined with an FT-IR Bruker FS 113V spectrophotometer for solutions in chloroform or as KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini 300 VT spectrometer (300 and 75.5 MHz, respectively) operating in the Fourier transform mode using solutions in $CDCl_3$. ^{1}H NMR chemical shifts (δ, ppm) are given relative to tetramethylsilane as the internal standard and coupling constants (*J*) are given in Hz. ^{13}C NMR shifts were referenced to solvent signal at δ 77.00. The DEPT technique was used for the assignment of multiplicity of carbon signals in ^{13}C NMR spectra. The additivity rules and comparison with data[9,31,32] reported for compounds of similar structure were helpful in signal assignment. Electron impact (ionization energy of 70 eV) mass spectra were recorded with an AMD 402 or AMD 604 spectrometer. Solvents were dried and distilled according to the standard procedures. The progress of reactions and purity of compounds were monitored by thin layer chromatography (TLC) using precoated aluminum-backed silica plates (Merck, No. 5554). Compounds were visualized using a 10% sulfuric acid followed by heating. Silica gel 60 (Merck 70–230 mesh, No. 7734) was used for flash chromatography. The usual work-up means dilution of the reaction mixture with hexane (diethyl ether or benzene) and successive washing of the organic layer with water, 5% $NaHCO_3$ solution, water, drying over anhydrous $MgSO_4$ and evaporation of the solvent under reduced pressure. Samples for elemental analysis were dried over phosphorus pentoxide under reduced pressure at 77 °C.

TABLE I
^{13}C NMR chemical shifts of 25-hydroxycholestane derivatives

Carbon	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	37.23	37.62	35.58	34.21	35.67	35.09	33.79	155.27
2	29.68	26.84	33.96	33.97	33.94	33.79	31.80	126.63
3	71.81	210.37	199.68	199.41	199.60	198.82	216.59	185.59
4	41.13	48.33	123.76	125.29	123.76	127.69	48.71	128.07
5	140.78	138.56	171.68	160.94	171.61	163.97	149.66	162.24
6	121.72	122.89	32.91	202.11	32.90	28.31	119.84	28.25
7	31.63	31.88	32.01	29.72	32.01	31.81	32.16	33.08
8	31.88	31.76	35.63	35.52	35.60	35.39	31.28	35.61
9	50.11	49.12	53.78	50.92	53.79	54.26	48.96	53.01
10	36.48	36.88	41.05	41.05	38.57	39.02	37.13	43.42
11	21.05	21.31	20.99	20.91	20.99	21.13	21.37	23.22
12	39.77	39.64	38.57	39.13	39.61	39.78	39.81	39.65
13	42.33	42.37	42.39	42.56	42.39	42.46	42.44	42.73
14	56.74[a]	56.56[a]	56.14[a]	56.49[a]	56.04[a]	55.99[a]	56.79[a]	56.05[a]
15	24.25	24.22	24.13	23.98	24.12	24.19	24.25	24.41
16	28.20	28.19	28.15	28.04	28.14	27.80	28.36	27.83
17	56.19[a]	56.15[a]	55.84[a]	55.99[a]	55.86[a]	56.06[a]	56.09[a]	55.54[a]
18	11.84	11.88	11.93	11.97	11.91	12.07	12.03	10.59
19	19.36	19.14	17.35	17.54	17.34	17.28	19.43	19.24
20	35.68	35.67	35.63	35.61	35.67	35.77	35.79	35.76
21	18.56	18.56	18.49	18.57	18.57	18.68	18.76	18.64
22	36.18	36.13	36.09	36.07	36.36	36.44	36.49	36.40
23	20.42	20.40	20.39	20.47	20.74	20.85	20.85	20.86
24	49.92	41.09	39.61	46.78	44.36	44.42	44.45	44.40
25	82.58	82.54	82.52	82.52	71.02	71.05	71.07	71.01
26	26.02	26.03	26.04	26.10	29.19[b]	29.28[b]	29.23[b]	29.29[b]
27	26.02	26.03	26.04	26.10	29.32[b]	29.43[b]	29.45[b]	29.43[b]
4-Me	–	–	–	–	–	11.16	–	12.17
4α-Me	–	–	–	–	–	–	30.28	–
4β-Me	–	–	–	–	–	–	27.33	–
CH_3CO_2-	22.46	22.47	22.47	22.55	–	–	–	–
CH_3CO_2-	170.57	170.53	170.53	170.43	–	–	–	–

[a,b] These signals may be interchanged.

Enol-Acetylation–Bromination–Dehydrobromination of Ketone **1**

To a solution of acetylating mixture (10 ml, prepared by mixing acetic anhydride (5.96 ml), perchloric acid (0.04 ml) and ethyl acetate (added to the total volume of 100 ml)), compound **1** (272 mg, 0.63 mmol) was added and the mixture was stirred for 5 h. The usual work-up gave the crude enol acetate (280 mg, 93%) as an oil. This sample was dissolved in a solution of sodium acetate (60 mg) in acetic acid (5 ml) and a solution of bromine (0.2 ml, 3.90 mmol) in acetic acid (5 ml) was added. The mixture was stirred for 1 h, poured into ice-water and the product extracted with hexane. The usual work-up gave a mixture of **2** and **3** (252 mg, 83% yield). ^{1}H NMR: 0.69 s, 3 H (Me-18); 0.92 d, 3 H (Me-21, J = 6.6); 1.08 s, 3 H (Me-19); 2.05 s, 3 H (OAc); 4.02 m, 2 H (H-24); 4.66 m, 1 H (H-C-Br); 4.98 d, 1 H (H-C-Br, J = 12.3).

The crude mixture of bromo ketones (240 mg) was dissolved in DMF (5 ml) containing lithium chloride (270 mg). Lithium carbonate (30 mg, 0.4 mmol) was added and the mixture was refluxed for 1 h. The usual work-up gave a residue (213 mg), which was crystallized from MeOH to give pure enone **4** (97 mg) in 48% yield. M.p. 125–128 °C; ref.[23] gives m.p. 123–125 °C. ^{1}H NMR spectra are in accordance with ref.[23].

The mother liquors were chromatographed on a silica gel column with chloroform as eluent to give pure enone **5**. M.p. 80–85 °C (MeOH). IR ($CHCl_3$): 2941, 2869, 2248, 1729, 1672, 1446, 1375, 1250, 1035, 783. ^{1}H NMR: 0.69 s, 3 H (Me-18); 0.93 d, 3 H (Me-21, J = 6.6); 1.19 s, 3 H (Me-19); 2.05 s, 3 H (OAc); 2.77 t, 1 H (H-4α, J = 16.5); 4.02 m, 2 H (H-24); 5.89 d, 1 H (H-1, J = 10.1); 6.84 d, 1 H (H-2, J = 10.1). ^{13}C NMR: 12.1, 18.6, 20.9, 21.1, 22.4, 24.3, 25.3, 26.0, 26.5, 28.1, 31.9, 35.2, 35.4, 38.7, 39.1, 39.9, 41.0, 42.7, 46.3, 55.7, 56.1, 65.0 (C-24); 126.8 (C-2); 161.5 (C-1); 171 ($OCOCH_3$); 200.6 (C-3). EI MS, *m/z* (%): 400 (20, M^+), 358 (1), 271 (16), 134 (33). EI HR MS for $C_{26}H_{40}O_3$ calculated: 400.29854; found: 400.29776. For $C_{26}H_{40}O_3$ (400.6) calculated: 77.95% C, 10.06% H; found: 78.08% C, 10.09% H.

25-Acetoxycholesterol (**9**)

To a solution of diacetate[29] **8** (532 mg, 1.1 mmol) in methanol (170 ml) a saturated solution of potassium carbonate in methanol (15 ml) was added and the mixture was stirred at room temperature for 18 h. The usual work-up gave compound **9** (485 mg, 99%, pure on TLC) as an oil. IR ($CHCl_3$): 3439, 1720, 1210, 1208. ^{1}H NMR: 0.68 s, 3 H (Me-18); 0.92 d, 3 H (Me-21, J = 6.6); 1.01 s, 3 H (Me-19); 1.42 s, 6 H (Me-26,27); 1.97 s, 3 H (AcO-25); 3.52 m, 1 H (H-3α); 5.36 bs, 1 H (H-6). For ^{13}C NMR, see Table I. EI MS, *m/z* (%): 444 (14, M^+), 384 (100), 369 (33), 271 (33). EI HR MS for $C_{29}H_{48}O_3$ calculated: 444.36035; found: 444.36134.

25-Acetoxycholest-5-en-3-one (**10**)

A) To a solution of alcohol **9** (35.5 mg, 0.08 mmol) in methylene chloride (2 ml) pyridinium chlorochromate (50 mg, 0.23 mmol) was added and the mixture was stirred at room temperature for 1 h. Anhydrous ether (2 ml) was added and stirring was continued for 10 min. The reaction mixture was filtered through Florisil (60–100 mesh) and the solvent was evaporated to give enone **10** (25.6 mg, 72%, pure on TLC). M.p. 100–105 °C (MeOH). IR ($CHCl_3$): 3530, 3306, 2948, 2870, 1720, 1677, 1456, 1368, 1271, 1197, 1019. ^{1}H NMR: 0.71 s, 3 H (Me-18); 0.92 d, 3 H (Me-21, J = 6.6); 1.19 s, 3 H (Me-19); 1.42 s, 6 H (Me-26,27); 1.97 s, 3 H (Ac-O25); 2.30 bd, 1 H (H-2, J = 13.1); 2.49 dt, 1 H (H-2, J_1 = 13.5, J_2 = 10.8, J_3 =

5.8); 2.82 dd, 1 H (H-4, J_1 = 16.5, J_2 = 1.9); 3.29 bd, 1 H (H-4, J = 16.2); 5.35 bs, 1 H (H-6). For ^{13}C NMR, see Table I. EI MS, *m/z* (%): 442 (10, M^+), 382 (100), 298 (26), 269 (60), 229 (28), 55 (43). EI HR MS for $C_{29}H_{46}O_3$ calculated: 442.34470; found: 442.34506. For $C_{29}H_{46}O_3$ (442.7) calculated: 78.68% C, 10.47% H; found: 78.74% C, 10.52% H.

B) To a solution of compound **9** (94 mg, 0.22 mmol) in methylene chloride (5 ml) anhydrous calcium carbonate (105 mg, 1.05 mmol) was added and the mixture was stirred for 15 min. Pyridinum chlorochromate (186 mg, 0.86 mmol) was added and stirring was continued for 1 h. Work-up as above gave enone **10** (72.1 mg, 77%).

25-Acetoxycholest-4-ene-3,6-dione (**12**)

To a solution of alcohol **9** (108 mg, 0.25 mmol) in methylene chloride (5 ml) anhydrous calcium carbonate (100 mg, 1.0 mmol) was added and the mixture was stirred for 15 min. Pyridinium chlorochromate (200 mg, 0.92 mmol) was added and stirring was continued for 5 h. Work-up as above gave enedione **12** (68.6 mg, 66%). M.p. 175–180 °C (MeOH–$CHCl_3$). IR ($CHCl_3$): 3365, 2946, 2869, 1733, 1465, 1368, 1255. ^{1}H NMR: 0.73 s, 3 H (Me-18); 0.93 d, 3 H (Me-21, J = 6.6); 1.17 s, 3 H (Me-19); 1.42 s, 6 H (Me-26,27); 1.97 s, 3 H (AcO-25); 2.69 dd, 1 H (H-2, J_1 = 15.5, J_2 =3.7); 6.18 s, 1 H (H-4). For ^{13}C NMR, see Table I. EI MS, *m/z* (%): 396 (7, M^+ – OAc), 382 (4), 283 (7), 137 (10), 69 (20), 43 (100). EI HR MS for $C_{27}H_{41}O_2$ calculated: 396.30283; found: 396.30389. For $C_{29}H_{44}O_4$ (456.7) calculated: 76.27% C, 9.71% H; found: 76.48% C, 9.81% H.

25-Acetoxycholest-4-en-3-one (**11**)

A) To a refluxed solution of alcohol **9** (50 mg, 0.11 mmol) in toluene (1.85 ml) and cyclohexanone (0.25 ml, 2.41 mmol) mixture a solution of aluminum isopropoxide (46 mg, 0.22 mmol) in toluene (1 ml) was added. After 1 h an additional portion of aluminum isopropoxide (36 mg, 0.18 mmol) was added and stirring was continued for 1 h. The cooled reaction mixture was poured on ice/H_2SO_4 (1 ml) and the product was extracted with ether. The usual work-up gave the crude product (89 mg) which was chromatographed on a silica gel column (2 g, hexane–ethyl acetate) to give enone **11** (32.2 mg, 64%). M.p. 154–158 °C (MeOH–$CHCl_3$). IR ($CHCl_3$): 3018, 2872, 1719. ^{1}H NMR: 0.70 s, 3 H (Me-18); 0.91 d, 3 H (Me-21, J = 6.6); 1.18 s, 3 H (Me-19); 1.42 s, 6 H (Me-26,27); 1.97 s, 3 H (OAc-25); 5.72 s, 1 H (H-4). For ^{13}C NMR, see Table I. EI MS, *m/z* (%): 442 (2.8, M^+), 382 (100), 340 (18), 298 (28), 269 (45), 124 (59). EI HR MS for $C_{27}H_{43}O$ calculated: 382.32358; found: 382.32367. For $C_{29}H_{46}O_3$ (442.7) calculated: 78.68% C, 10.47% H; found: 78.88% C, 10.44% H.

B) To a solution of enone **10** (120 mg, 0.27 mmol) in ethanol (95%, 7 ml) oxalic acid (30.2 mg, 0.33 mmol) was added and the mixture was stirred at reflux for 1 h. After cooling the product was extracted with ether. The usual work-up gave the crude product (109 mg, 91%), which was chromatographed on a silica gel column (4 g, hexane–ethyl acetate) to give enone **11** (50 mg, 47%). ^{1}H NMR spectrum was identical with that of sample described above.

25-Hydroxycholest-4-en-3-one (**13**)

To a refluxing solution of diol **7** (1.47 g, 3.7 mmol) in toluene (30 ml) and cyclohexanone (5 ml, 48.2 mmol) mixture a solution of aluminum isopropoxide (800 mg, 3.8 mmol) in toluene (15 ml) was added dropwise during 30 min and the mixture was refluxed for additional

40 min. The cooled reaction mixture was poured on ice/H_2SO_4 and the product was extracted with hexane–ether. The usual work-up gave crude product (1.8 g), which was chromatographed on a silica gel column (10 g, benzene) to give pure **13** (1.2 g, 86%). M.p. 150–152 °C (MeOH–hexane); ref.[21] gives m.p. 147–148 °C. IR (KBr): 3470, 1657, 1610. ^{1}H NMR spectrum in agreement with ref.[33]

Methylation of 25-hydroxycholest-4-en-3-one (**13**)

To a boiling solution of potassium *tert*-butoxide in *tert*-butanol (potassium (230 mg, 5.9 mmol) and *t*-BuOH (40 ml)) a solution of enone **13** (497 mg, 1.2 mmol) in *tert*-butanol (25 ml) was added dropwise over 1 h. The solution of methyl iodide (3.8 ml, 61.04 mmol) in *tert*-butanol (18 ml) was added dropwise over 1.5 h and the resulting mixture was refluxed for 30 min. After cooling, the reaction mixture was acidified with 1 M HCl and the solvent was evaporated. The crude product was extracted with benzene, the organic layer was washed with $NaHSO_3$, brine and water, dried with $MgSO_4$ and evaporated to give crude product (505 mg) which was chromatographed on dry silica gel column (3 g, benzene) to give 4-methyl-25-hydroxycholest-4-en-3-one **14** (168 mg, 34%) and 4,4-dimethyl-25-hydroxycholest-5-en-3-one **15** (130 mg, 25%). Additionally, other fractions afforded slightly impure compound **14** (33 mg) and **15** (46 mg).

Compound **14**: M.p. 151–154 °C (MeOH–$CHCl_3$). IR (KBr): 3567, 2964, 1656, 1601, 1468, 1376, 1332, 1310, 1246, 1143, 1027, 918. ^{1}H NMR: 0.71 s, 3 H (Me-18); 0.92 d, 3 H (Me-21, J = 6.5); 1.16 s, 3 H (Me-19); 1.21 s, 6 H (Me-26,27); 1.77 d, 3 H (Me-4, J = 1.1); 2.74 dt, 1 H (H-6, J_1 = 14.8, J_2 = 3.3). For ^{13}C NMR, see Table I. EI MS, *m/z* (%): 414 (6.5, M^+), 396 (40), 381 (11), 283 (21), 138 (81), 59 (100). EI HR MS for $C_{28}H_{46}O_2$ calculated: 414.34979; found: 414.34699. For $C_{29}H_{46}O_2$ (414.7) calculated: 81.10% C, 11.18% H; found: 81.19% C, 11.08% H.

Compound **15**: M.p. 210–215 °C (MeOH–$CHCl_3$). IR (KBr): 3567, 2954, 2867, 1700, 1655, 1460, 1376, 1254, 1136, 1029, 915. ^{1}H NMR: 0.67 s, 3 H (Me-18); 0.85 s, 3 H (Me-19); 0.94 d, 3 H (Me-21, J = 6.3); 1.22 s, 6 H (Me-26,27); 1.23 s, 6 H (Me-4α and Me-4β); 2.39–2.62 m, 2 H (H-2); 5.56 m, 1 H (H-6). For ^{13}C NMR, see Table I. EI MS, *m/z* (%): 428 (23, M^+), 410 (17), 395 (12), 123 (100). EI HR MS for $C_{29}H_{48}O_2$ calculated: 428.36542; found: 428.3637. For $C_{29}H_{48}O_2$ (428.7) calculated: 81.25% C, 11.29% H; found: 81.20% C, 11.31% H.

Methylation of 25-Acetoxycholest-4-en-3-one (**11**)

To a boiling solution of potassium *tert*-butoxide in *tert*-butanol (potassium (35 mg, 0.90 mmol) and *t*-BuOH (6.3 ml)) a solution of enone **11** (74.6 mg, 0.16 mmol) in *tert*-butanol (3.5 ml) was added dropwise and the mixture was refluxed for 1 h. The solution of methyl iodide (0.56 ml, 8.9 mmol) in *tert*-butanol (6.3 ml) was added dropwise over 1 h. After 30 min the reaction mixture was cooled to room temperature, diluted HCl was added to adjust pH 6 and the solvent was evaporated under reduced pressure. Ether was added and the organic layer was washed with $NaHSO_3$, water and dried with $MgSO_4$. Evaporation of the solvent gave the crude product (69.6 mg, 90%) as a mixture of compounds **14** and **15** in 3:4 ratio and some traces of the substrate **11** (estimated from ^{1}H NMR spectrum by integration of signals at δ 2.74 and 2.50).

4-Methyl-25-hydroxycholesta-1,4-dien-3-one (**16**)

To a solution of enone **14** (71 mg, 0.17 mmol) in anhydrous dioxane (10 ml) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (200 mg, 0.88 mmol) was added and the mixture was stirred at reflux for 22 h. After cooling the solution was filtered through column of Al_2O_3 (neutral, 1 g) which was eluted with benzene. The crude product (66 mg, 92%) was chromatographed on a silica gel column (1.5 g, $CHCl_3$–hexane 40:1) to give enedione **16** (44.5 mg, 62%) as an oil and the substrate **14** (12.5 mg, 18%).

Compound **16**: IR ($CHCl_3$): 3023, 3016, 2941, 1657, 1618, 1223. ^{1}H NMR: 0.74 s, 3 H (Me-18); 0.92 d, 3 H (Me-21, J = 6.6); 1.21 s, 6 H (Me- 26,27); 1.25 s, 3 H (Me-19); 1.91 s, 3 H (Me-4); 2.85–2.26 m, 1 H (H-6); 6.25 d, 1 H (H-2, J = 10.1); 7.03 d, 1 H (H-1, J = 10.1). For ^{13}C NMR, see Table I. EI MS, *m/z* (%): 412 (11, M^+), 394 (45), 379 (6), 338 (13), 283 (18), 136 (87), 82 (97), 55 (100). EI HR MS for $C_{28}H_{44}O_2$ calculated: 412.33414; found: 412.33621.

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