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Inhibitors of sterol synthesis. Synthesis and spectral properties of 3β-hydroxy-5α-cholestan-15-one and its 14β-epimer and their effects on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity

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Abstract

3β-Hydroxy-5α-cholestan-15-one (**2a**) and its 14β-epimer **2b** were prepared from 3β-acetoxy-5α-cholest-8(14)-ene (**3**). Hydroboration of **3** at 45–50°C gave a mixture of 5α,14α-cholestane-3β,15α-diol and 5α,14β-cholestane-3β,15β-diol, which were separated on silica gel as their 3β-*tert*-butyldimethylsilyl ethers **5a** and **5b**. Oxidation of **5a** with pyridinium chlorochromate, followed by desilylation with tetrabutylammonium fluoride gave **2a**. Analogous transformations of **5b** gave **2b** contaminated with **2a**. Desilylation of **5b** followed by oxidation with pyridinium chlorochromate resulted in a mixture composed mainly of 5α,14β-cholestane-3,15-dione and **2b**. Successive chromatographic separations on silica gel and reversed phase media gave **2b** of high purity. Compound **2a** was also prepared by lithium-ammonia reduction of 3β-hydroxy-5α-cholest-8(14)-en-15-one (96% yield) and by selective reduction of 5α-cholestane-3,15-dione with lithium tri-*tert*-butoxyaluminum hydride (90% yield). Isomers **2a** and **2b** were readily epimerized under acidic or basic conditions or under conditions used for gas chromatographic analysis. The purities of **2a** and **2b** were measured from nuclear magnetic resonance (NMR) spectra; chromatographic methods gave less reliable estimates of purity. NMR data also showed that ring C of the 14β sterols is predominantly in a chair conformation. The effects of **2a** and **2b** on the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase have been studied in Chinese hamster ovary cells.

Keywords: 15-Ketosterols; Liquid-ammonia reduction; NMR; Conformational analysis

1. Introduction

3β-Hydroxy-5α-cholest-8(14)-en-15-one (**1**) (Fig. 1) is a potent inhibitor of sterol synthesis in cultured mammalian cells and lowers the levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity and of two other enzymes involved in the formation of mevalonic acid [1–8]. The $\Delta^{8(14)}$-15-ketosterol is one of the most potent oxysterols in the lowering of reductase activity and in the affinity of binding to an oxysterol binding protein that has been reported to play a role in the

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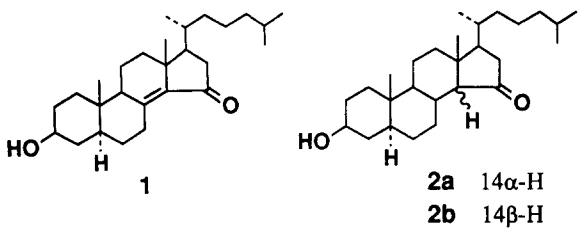

Fig. 1. Structure of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**1**) and saturated analogs of **1**.

regulation of reductase activity in cultured cells [4]. In the present study we sought to extend structure-activity relationships for **1** and specifically to evaluate the importance of the $\Delta^{8(14)}$ double bond of **1** in its action in lowering reductase activity. Described herein is an improved synthesis of 3β-hydroxy-5α-cholestan-15-one **2a**, the first preparation of its 14β isomer **2b** and the effects of these two saturated 15-ketosterols on the levels of HMG-CoA reductase activity in CHO-K1 cells.

2. Experimental procedures and results

2.1. Materials and methods

General experimental procedures for melting points (mp), infrared (IR) spectra, ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra, radioactivity measurements, thin-layer chromatography (TLC), medium pressure liquid chromatography (MPLC; 20-ml fraction volumes), reversed phase high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), capillary GC and high-resolution MS (electron impact mode) have been described previously [9] or are given in footnotes to Table 1. The full-scan high resolution MS of *tert*-butyldimethylsilyl (TBDMS) ethers **5a**, **5b**, **6a** and **6b** showed negligible abundance for M^+ and data for M^+ were acquired in a separate experiment. Optical rotations were measured on a JASCO DIP-4 digital polarimeter at room temperature (~23°C) in $CHCl_3$ solution. Solvent systems (SS) for TLC were: SS-1, 5:95 ethyl acetate-hexane; SS-

Table 1
Chromatographic behavior of 3β-hydroxy-5α,14α-cholestan-15-one (**2a**) and its 14β epimer **2b**[a]

Method	2a	2b
TLC (40% ethyl acetate in hexane)	0.59[b]	0.59[b]
TLC (50% ether in benzene)	0.49[b]	0.49[b]
HPLC (methanol)[c]	6.4[c]	5.7[c]
HPLC (5% water in methanol)[c]	14.1[c] (98:2)	12.0[c] (<1:99)
GC (30-m DB-5 column, TMS ether)	17.8[d] (83:17)	15.0[d] (46:54)
GC (30-m DB-5 column, free sterol)	16.8[d] (77:23[e])	14.4[d] (46:54[e])
GC-MS[f] (15-m DB-5 column, free sterol)	9.4[g] (97:3)	8.7[g] (14:86)

[a]Analysis of samples of **2a** and **2b** of >99% purity by ^1H NMR. Values in parentheses after retention times indicate the observed ratio of **2a**:**2b**.

[b]R_f values from TLC on silica gel G plates (single component on heavily overloaded plates).

[c]Retention times (min) for reversed phase HPLC (250 mm × 4.6 mm column, 5-μm Spherisorb ODS-II column, 1 ml/min, UV detection at 210 nm).

[d]Retention times (min) for GC using a Shimadzu GC-9A chromatograph, splitless injection at 280°C, nitrogen carrier gas, flame ionization detection and a DB-5 column (30 m × 0.25 mm i.d.; 0.1 μm film thickness) held at 200°C for 3 min and increased to 280°C at 20°C/min.

[e]At an injector temperature of 200°C, ratios of **2a**:**2b** indicated somewhat less epimerization: **2a**, 83:17; **2b**, 37:63.

[f]GC-MS data: **2a**, *m/z* 402 (51), 387 (6), 384 (17), 369 (8), 330 (19), 289 (12), 276 (15), 271 (7), 261 (79), 253 (6), 234 (30), 217 (31), 216 (35), 209 (38), 108 (100); **2b**, *m/z* 402 (4), 384 (6), 289 (13), 271 (6), 209 (100), 107 (15); TMS ether of **2a**, *m/z* 474 (44), 459 (100), 384 (27), 369 (12), 345 (11), 333 (14), 327 (10), 216 (19), 209 (28), 107 (47); TMS ether of **2b**, 474 (9), 459 (13), 361 (21), 281 (14), 209 (100), 107 (13).

[g]Retention times (min) for GC-MS using falling needle injection at 280°C, helium carrier gas and a 15-m DB-5 column held at 200°C for 2 min and increased to 280°C at 10°C/min.

2, 1:9 ethyl acetate-hexane; SS-3, 3:7 ethyl acetate-hexane; SS-4, 4:6 ethyl acetate-hexane; SS-5, 1:1 ethyl acetate-hexane; SS-6, 5:95 ether-benzene; SS-7, 1:3 ether-benzene; SS-8, 1:1 ether-benzene; SS-9, 2:8 ethyl acetate-toluene. Methodology has also been described for nuclear Overhauser effect (NOE) and saturation difference spectra, NMR coupling constant measurements, force-field calculations, and spin simulation [10]. HMQC (heteronuclear multiple-quantum coherence) spectra were acquired in the phase-sensitive mode with ^{13}C decoupling using a ~0.8-s acquisition time, 0.5-s relaxation delay and digital resolution of ~0.5 Hz in f_2 and ~30 Hz in f_1. ^1H and ^{13}C NMR assignments were made from a combination of standard 1D and 2D spectra [10,11] in conjunction with ^1H and ^{13}C chemical shift comparisons [10,12,13] and substituent increments [11]. ^1H NMR stereochemical assignments were made by chemical shift comparisons [14] or by comparing observed coupling constants with those predicted from force-field calculations. The purity of sterol samples was estimated by TLC and ^1H NMR (500 MHz spectrum; methyl region and δ 2.5–7.0 region).

As described previously [9], cell culture experiments were performed by successively incubating CHO-K1 cells in lipid-rich medium for 48 h and lipid-deficient medium for 18 h, then incubating for 4 h in fresh lipid-deficient medium containing sterols, harvesting and assaying for HMG-CoA reductase activity.

Borane-methyl sulfide, pyridinium chlorochromate, *tert*-butyldimethylsilyl chloride, lithium tri-*tert*-butoxyaluminum hydride and Florisil were purchased from Aldrich Chemical Company (Milwaukee, WI). The preparation of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**1**) [15], 5α,14α-cholestane-3β,15α-diol (**4a**) [16] and trimethylsilyl (TMS) ether derivatives [9] has been described. Samples of 5α,14α-cholestane-3,15-dione (**7a**) and its 14β epimer **7b**, prepared as described previously [16], showed respective purities of >99% and ≥98% (containing 1–2% **7a**) by ^1H NMR analysis. 3β-Acetoxy-5α-cholest-8(14)-ene (**3**) was prepared by hydrogenation-isomerization of 7-dehydrocholesterol acetate (10 g) overnight under H_2 (1 atm) in ethyl acetate-acetic acid (4:1, 250 ml) containing 5% palladium on carbon (2.5 g). Filtration followed by washing with saturated $NaHCO_3$ and water gave a crude product that was recrystallized from ethyl acetate-methanol to give 8.45 g of **3**, mp 76.5–77.5°C, lit. mp 78°C [17]. Structures of compounds **3–8** are shown in Fig. 2.

2.2. *Mixture of 5α,14α-cholestane-3β,15α-diol* (**4a**) *and 5α,14β-cholestane-3β,15β-diol* (**4b**) *from hydroboration of 3β-acetoxy-5α-cholest-8(14)-ene* (**3**)

To a solution of **3** (5 g; 11.7 mmol) in tetrahydrofuran (50 ml) was added neat borane-methyl sulfide (10 M solution, 15 ml) and the resulting solution was stirred at 45–50°C for 6 h under nitrogen. The mixture was cooled to 4°C and ice was cautiously added followed by water and ether (500 ml). After separation of the layers, the organic phase was washed with water (3 × 500 ml), dried over Na_2SO_4 and evaporated to a yellowish oil. The residue was dissolved in tetrahydrofuran (200 ml) and cooled to 4°C. Solutions of 20% aqueous NaOH (75 ml) and 30% H_2O_2 (75 ml) were added slowly. The mixture was stirred for 1 h at 4°C and 3 h at room temperature. Ether (500 ml) was added and the separated organic phase was washed with 10% aqueous sodium sulfite (2 × 200 ml) and water (3 × 500 ml). Evaporation of the organic phase gave a white solid (4.69 g) that was subjected to MPLC (460 mm × 25 mm i.d. column; 100 g silica gel, 230–400 mesh; elution with 20% ethyl acetate in hexane). Evaporation of fractions 112–186 gave a mixture of diols **4a** and **4b** (3.71 g) in a ratio of 22:78 as judged by ^1H NMR (500 MHz).

2.3. *3β-tert-Butyldimethylsilyloxy-5α,14α-cholestan-15α-ol* (**5a**) *and 3β-tert-butyldimethylsilyloxy-5α,14β-cholestan-15β-ol* (**5b**)

To a solution of the 22:78 mixture of diols **4a** and **4b** (2 g, 4.94 mmol) in benzene (75 ml) and dimethylformamide (25 ml) was added imidazole (2.02 g, 29.7 mmol), and traces of moisture were removed by refluxing with a Dean-Stark apparatus. To the dried solution was added *tert*-butyldimethylsilyl chloride (1.119 g, 7.42 mmol, 1.5 equiv.) and the resulting mixture was heated at 70°C for 1 h. After TLC analysis indicated that the

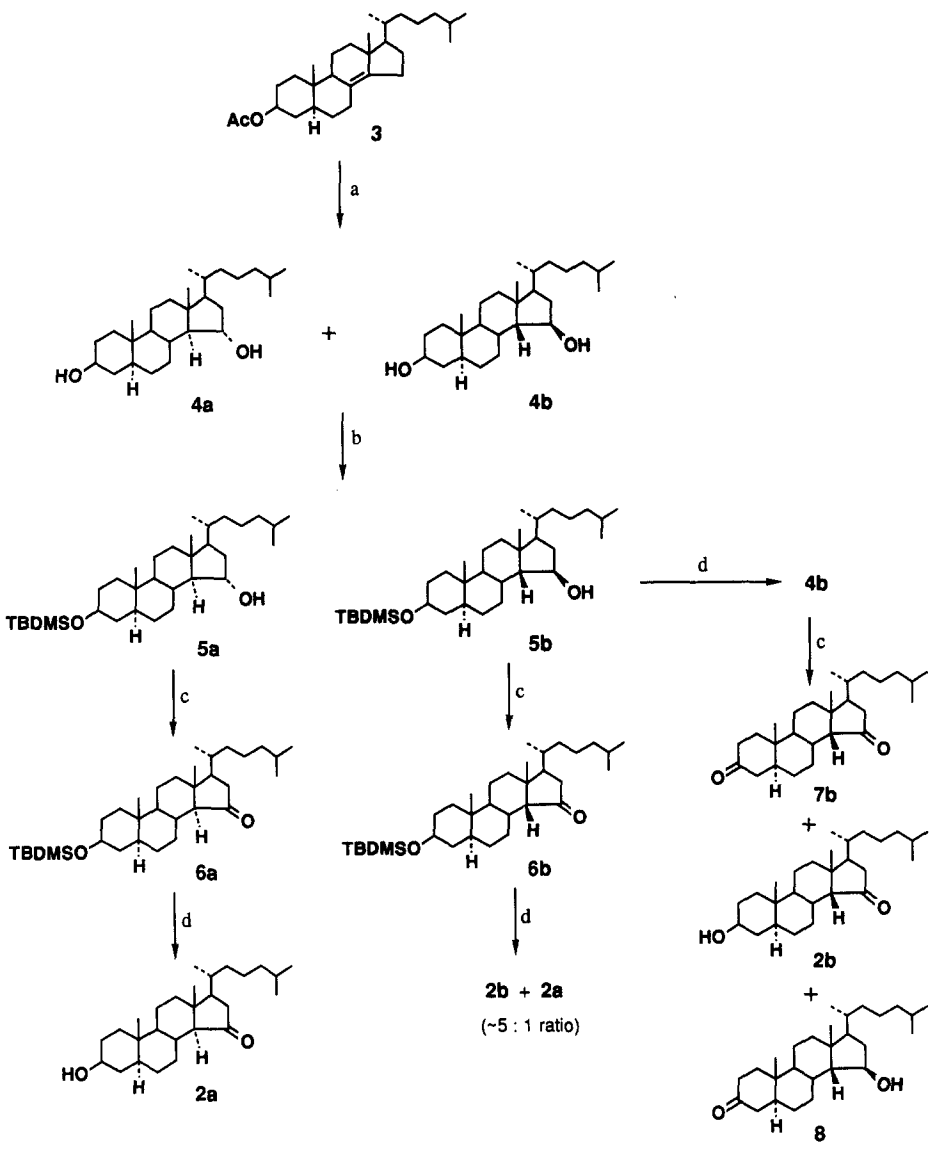

Fig. 2. Chemical synthesis of 3β-hydroxy-5α,14β-cholestan-15-one (**2b**) and other saturated 15-oxygenated sterols. Reagents and conditions: (a) borane-dimethyl sulfide, 45–50°C; NaOH-H_2O_2; (b) *tert*-butyldimethylsilyl chloride, imidazole, dimethylformamide, 70°C; (c) pyridinium chlorochromate, NaOAc; (d) pTsOH, acetone.

reaction was complete, the mixture was poured over 5% $NaHCO_3$ (100 ml) and extracted with ethyl acetate (2 × 250 ml). The combined organic layers were washed with water (3 × 250 ml), dried over Na_2SO_4 and evaporated to a white solid (2.32 g). The crude product was purified by MPLC (960 mm × 10 mm i.d. column; 38 g silica gel, 230–400 mesh; elution with 1% ethyl acetate in hexane). Evaporation of fractions 48–81 gave the 15β-hydroxy isomer **5b** as a foamy solid (1.681 g; 52% yield from **3**), showing ≥99% purity by ^1H NMR: mp 56–57°C; TLC, single component in SS-2 (R_f 0.63) and SS-6 (R_f 0.81); $[\alpha]_D^{23}$ +25.8°C (*c* 0.7, $CHCl_3$); IR ν_{max} 3400, 2953, 2930, 2857, 1466,

Table 2
^{1}H NMR chemical shifts for saturated 15-oxygenated sterols[a,b]

	3β-OH		3β-RSiO		3β-RSiO		3β-OH		3-one	
	15α-OH	15β-OH	15α-OH	15β-OH	15-one		15-one		15-one	
	4a	**4b**	**5a**[c]	**5b**[c]	**6a**[c]	**6b**[c]	**2a**	**2b**	**7a**	**7b**
H-1α	0.98	0.96	0.93	0.92	0.93	0.91	0.98	0.95	1.35	1.32
H-1β	1.71	1.74	1.66	1.70	1.65	1.65	1.70	1.69	2.01	1.98
H-2α	1.80	1.80	1.68	1.67	1.68	1.64	1.81	1.77	2.30	2.27
H-2β	1.40	1.40	1.43	1.43	1.44	1.39	1.40	1.36	2.39	2.34
H-3α	3.59	3.60	3.55	3.55	3.54	3.54	3.59	3.59		
H-4α	1.57	1.58	1.44	1.46	1.44	1.47	1.57	1.59	2.09	2.13
H-4β	1.29	1.30	1.33	1.33	1.32	1.32	1.29	1.28	2.26	2.24
H-5α	1.12	1.12	1.08	1.08	1.08	1.17†	1.12	1.21†	1.54	1.62
H-6α	1.28†	1.30	1.28†	1.29	1.28†	1.28	1.29†	1.31	1.36†	1.37
H-6β	1.28†	1.26	1.27†	1.24	1.28†	1.18†	1.29†	1.20	1.36†	1.25
H-7α	1.12	1.48	1.11	1.46	0.82	2.46	0.85†	2.47	0.87	2.51
H-7β	1.90	1.59	1.88	1.58	2.65	1.40	2.66	1.41	2.71	1.45
H-8β	1.57	1.70	1.56	1.69	1.69	1.63	1.70	1.64	1.75	1.69
H-9α	0.68	0.92	0.66	0.90	0.61	0.86	0.64	0.89	0.74	1.00
H-11α	1.51	1.45	1.50	1.44	1.54	1.48	1.55	1.49	1.59	1.53
H-11β	1.28	1.20†	1.27†	1.18†	1.26	1.20†	1.27	1.22	1.39†	1.31
H-12α	1.20	1.18	1.19	1.17†	1.34	1.16†	1.35	1.16†	1.37†	1.17†
H-12β	1.91	1.18	1.91	1.17†	2.11	1.33	2.11	1.33	2.15	1.37
H-14	1.04	1.51	1.03	1.51	1.66	2.11	1.67	2.12	1.70	2.15
H-15	3.94	4.28	3.94	4.27						
H-16α	1.71	2.29	1.71	2.28	2.42	2.32	2.42	2.32	2.44	2.33
H-16β	1.88	1.37	1.88	1.37	1.77	2.15	1.77	2.16	1.79	2.17
H-17α	1.40	1.40	1.40	1.40	1.55	1.64	1.55	1.64	1.57	1.66
H-18	0.684	1.002	0.678	0.995	0.733	1.149	0.739	1.156	0.768	1.183
H-19	0.820	0.791	0.810	0.779	0.800	0.745	0.811	0.758	1.016	0.958
H-20	1.33†	1.67	1.34	1.67	1.47	1.91	1.47	1.91	1.48	1.92
H-21	0.893	0.845	0.891	0.843	0.980	0.860	0.981	0.863	0.990	0.871
H-22R	1.31†	1.38	1.32†	1.38	1.28	1.34	1.28	1.34	1.29	1.33
H-22S	1.00	1.08	1.00	1.08	1.03	0.98	1.03	0.98	1.04	0.99
H-23R	1.31†	1.38	1.32†	1.38	1.34	1.39	1.35	1.39	1.35	1.40
H-23S	1.13†	1.17	1.12†	1.18†	1.18	1.19	1.18	1.19	1.18	1.20
H-24	1.09	1.14	1.08	1.13	1.09	1.10	1.09	1.10	1.09	1.11
H-24	1.14†	1.18	1.14†	1.17†	1.14	1.17	1.14	1.16†	1.15	1.17
H-25	1.51	1.52	1.51	1.52	1.51	1.50	1.51	1.51	1.52	1.51
H-26	0.859	0.867	0.858	0.866	0.861	0.863	0.861	0.864	0.864	0.868
H-27	0.863	0.870	0.862	0.869	0.865	0.866	0.865	0.867	0.868	0.872

[a]Data from ^{1}H spectra at 500 MHz in $CDCl_3$ solution at 27°C and a concentration of 0.01–0.2 M (referenced to internal $(CH_3)_4Si$).
[b]Estimated accuracy ±0.01 ppm except for values marked by † (±0.02 ppm).
[c]3β-*tert*-Butyldimethylsilyl ethers (labelled 3β-RSiO) also showed singlets at δ 0.880 and 0.046 (all ±0.004 ppm).

1383, 1370, 1252, 1090, 1074, 870, 835, 774 cm^{-1}; high resolution MS, *m/z* 518.4554 (M^+, calcd. for $C_{33}H_{62}O_2Si$, 518.4519), 461.3838 (100, M-C_4H_9), 443.3719 (15, M–C_4H_9–H_2O), 367.3373 (2, $C_{27}H_{43}$), 107.0857 (7, C_8H_{11}); ^{1}H NMR, Tables 2 and 3; ^{13}C NMR, Table 4.

Evaporation of fractions 96–124 gave the 15α-hydroxy isomer **5a** as a colorless solid (498 mg; 15% yield from **3**), showing ≥99% purity by NMR: mp 84–85°C; TLC, single component in SS-2 (R_f 0.56) and SS-6 (R_f 0.74); $[\alpha]_D^{23}$ +38.9° (*c* 0.7, $CHCl_3$); IR ν_{max} 3360, 2951, 2932, 2855, 1466,

Table 3
^{1}H-^{1}H NMR coupling constants for saturated 15-oxygenated sterols[a]

	4a	4b	5a	5b	6a	6b	2a	2b	7a	7b
1α-1β	13.1	13.1		13.2†	13.2†		13.0†	13.2	13.2	13.2
1α-2α	3.8	3.7		4.1†	4.0†		3.7	4.0	5.2	5.2
1α-2β	14.0†	13.7		13.8†	13.8†		13.7	13.9†	13.6	13.8
1β-2α	3.5†	3.3	3.7†		3.6†		3.0†	3.3†	2.3	2.5
1β-2β	3.5†	3.7	3.7†		3.6†		4.0	3.9	6.6	6.4
2α-2β		12.7					12.7	12.8†	15.5	15.5
2α-3α	4.9†	5.0	4.8†	4.7†	4.8†	4.6†	4.8†	4.8		
2β-3α	11.1	11.3	11.0	10.9	10.8	10.9	11.1	11.0		
3α-4α	4.9†	5.0	4.8†	4.7†	4.8†	4.7	4.8†	4.8		
3α-4β	11.0	11.0	10.7	10.9	10.7	10.8	11.0	11.0		
4α-4β	12.4†	12.4†	12.6†	12.5	12.7†	12.5	12.5	12.1†	15.0	15.5
4α-5α		2.8		3.4†		2.3†		2.2†	3.9	4.3
4β-5α	12.4†	12.4†	12.6†	12.6	12.7†	12.6†	12.5	12.1†	13.9	13.6
5α-6α		3.5†		3.4†						3.1
5α-6β		~12.2‡		11.5						12.1
6α-6β		12.4†		12.6†						13.3†
6α-7α		4.5		4.7		4.6†				4.5
6α-7β		3.3†		3.4†	3.3†		3.2†			2.7
6β-7α		12.4†		12.5†		12.8†		~12.9‡		13.3†
6β-7β		3.8		3.4†	3.3†		3.2†			4.1
7α-7β		13.0		12.8	13.1	12.8†	13.1	~12.9‡		13.4
7α-8β		12.6†		12.0†	10.8†	12.5†	10.6†	12.2†		12.4
7β-8β		3.3†		3.5†	3.6	4.2†	3.6			4.2
8β-9α	10.6	11.8†	10.6	12.0†	10.2	12.1†	10.1	11.8†		11.9
8β-14	10.9	4.4	10.9	4.5	11.0†	4.2	10.4	4.4		4.6†
9α-11α	4.2	4.7†	4.2		4.0		4.0	3.5		3.5
9α-11β	10.1		11.9		12.2		12.2	11.8†		11.9
11α-11β					13.2†		13.2†	12.7†		13.2†
11α-12α	4.0				13.3†			3.4†		3.5†
11α-12β					3.3†		3.0†	3.4†		3.5†
11β-12α	12.9†				3.5†		13.2†			13.2†
11β-12β					3.3†		3.6			3.3†
12α-12β	12.9†				12.3		12.2			
14-15	9.0	8.6	8.9	8.3						
15-16α	3.3	8.2†	3.4	8.7†						
15-16β	9.8†	5.8†	9.3	5.1†						
16α-16β	14.3	13.6	14.3	13.4	18.8	19.8	18.9	19.8	18.8	19.9
16α-17α	8.7	9.0†	8.7	~9‡	8.7	10.1	8.7	10.1	8.8	10.1
16β-17α	9.8†	5.8	10.3	5.9†	9.6	3.1	9.7	3.1	9.5	3.3
17α-20		3.8†	9.9†	4.0†	10.3	2.7†	10.2	2.9		2.8
20-21	6.4	6.7		6.7	6.5	6.8	6.5	6.8	6.5	6.8
20-22R		2.2†			2.5	2.7†	2.6	2.6†	2.7	2.7†
20-22S		10.6†			8.7	11.0	8.6	11.0	8.7	11.0
22-22					12.5		12.6†	~12.5‡	12.5	
22R-23R								~10‡	10.4	
22R-23S					5.0†			~5‡	4.9	
22S-23R					4.1		4.1†	~4‡	4.0	
22S-23S					9.9		9.9†	9‡	10.1	
23-23					11.6				12.0†	
23R-24R					5.2		5.3	5.1		5.2
23R-24S					9.6†		9.8			9.9
23S-24R					10.2†		10.6	10.9	10.3†	10.8
23S-24S					4.8†		4.9		5.3†	4.9

Table 3 (*Continued*)

	4a	4b	5a	5b	6a	6b	2a	2b	7a	7b
24-24					13.1		13.4	13.2		13.2
24R-25	6.7†	6.6†	6.6†	6.6†	6.3†	6.6†	6.5	6.6	6.6†	6.6
24S-25	6.7†	6.6†	6.6†	6.6†	6.9†	6.6†	6.8	6.6†	6.6†	6.8
25-26	6.7	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
25-27	6.6	6.7	6.7	6.7	6.7	6.7	6.7	6.6	6.6	6.6

[a]Data obtained at 500.1 MHz in 0.02–0.1 M $CDCl_3$ solution (27°C). Accuracy is ca. ±0.2 Hz except for couplings marked by † (±0.5 Hz) or by ‡ (±1 Hz). Most compounds also showed $J_{2\alpha\text{-}4\alpha}$ of ~2.1 Hz and the 14β-15-ketosterols showed $J_{14\beta\text{-}16\beta}$, ~2.1 Hz and $J_{12\beta\text{-}14\beta}$, ~2.2 Hz. H-24R is assumed to be the upfield C-24 proton.

Table 4
^{13}C NMR chemical shifts for saturated 15-oxygenated sterols[a]

	3β-OH		3β-RSiO		3β-RSiO		3β-OH		3-one	
	15α-OH	15β-OH	15α-OH	15β-OH	15-one		15-one		15-one	
	4a	4b	5a[b]	5b[b]	6a[b]	6b[b]	2a	2b	7a	7b
C-1	36.99	36.89	37.18	37.08	37.14	36.90	36.94	36.67	38.42	37.95
C-2	31.43	31.27	31.90	31.74	31.87	31.69[c]	31.38	31.13	38.02	37.86
C-3	71.19	71.29	72.04	72.18	72.00	72.10	71.13	71.11	211.67	211.69
C-4	38.10	38.11	38.58	38.59	38.49	38.65	38.03	38.12	44.48	44.54
C-5	44.59	44.66	44.76	44.83	44.96	44.05	44.78	43.84	46.48	45.62
C-6	28.54	29.06	28.60	29.11	28.30	28.80	28.24	28.75	28.44	29.01
C-7	32.36	32.32	32.43	32.40	30.71	29.18	30.62	29.09	30.19	28.71
C-8	35.02	33.25	35.03	33.25	31.84	33.46	31.85	33.41	31.64	33.27
C-9	54.19	46.84	54.28	46.92	54.04	47.42	53.92	47.30	53.28	46.69
C-10	35.44	35.80	35.48	35.82	35.58	35.44	35.54	35.40	35.66	35.53
C-11	21.09	20.90	21.08	20.90	20.77	21.60	20.77	21.55	20.90	21.73
C-12	40.11	37.89	40.15	37.94	39.91	38.31	39.86	38.22	39.64	38.05
C-13	44.02	43.37	44.05	43.41	42.37	42.09	42.35	42.04	42.26	42.01
C-14	63.71	58.46	63.73	58.52	65.92	57.84	65.87	57.78	65.50	57.55
C-15	73.89	73.61	73.88	73.64	216.17	220.51	216.19	220.58	215.79	220.02
C-16	40.54	35.20	40.52	35.14	41.96	37.46	41.95	37.45	41.84	37.33
C-17	53.67	53.15	53.67	53.20	51.49	48.38	51.50	48.35	51.38	48.30
C-18	13.35	20.63	13.34	20.61	12.98	19.10	12.97	19.06	12.91	19.16
C-19	12.37	12.22	12.41	12.28	12.24	12.21	12.18	12.13	11.29	11.28
C-20	35.27	33.47	35.27	33.47	35.30	33.25	35.30	33.22	35.26	33.22
C-21	18.47	19.68	18.48	19.68	18.99	19.21	18.97	19.17	18.92	19.02
C-22	36.04	33.40	36.05	33.38	35.97	31.72[c]	35.96	31.71	35.88	31.70
C-23	23.70	25.36	23.69	25.37	23.72	25.50	23.72	25.46	23.66	25.45
C-24	39.43	39.43	39.43	39.42	39.27	39.26	39.25	39.23	39.19	39.24
C-25	27.97	28.03	27.98	28.04	27.92	28.00	27.91	27.96	27.86	27.97
C-26	22.54	22.56	22.55	22.57	22.49	22.50	22.48	22.46	22.44	22.47
C-27	22.78	22.73	22.79	22.74	22.74	22.71	22.72	22.67	22.69	22.58

[a]Chemical shifts referenced to the $CDCl_3$ signal at 77.0 ppm. Data obtained at 75 MHz in $CDCl_3$ solution (22°C) at a concentration of 0.05–0.1 M.
[b]3β-*tert*-Butyldimethylsilyl ethers (labelled 3β-RSiO) also showed signals at δ 25.93, 18.25, −4.60 (all ±0.03 ppm).
[c]Assignments may be interchanged.

1383, 1252, 1096, 870, 835, 774 cm^{-1}; high resolution MS, *m/z* 503.4301 ($M-CH_3$, calcd. for $C_{32}H_{59}O_2Si$, 503.4284), 461.3833 (100, $M-C_4H_9$), 443.3719 (14, $M-C_4H_9-H_2O$), 367.3373 (4, $C_{27}H_{43}$), 107.0853 (6, C_8H_{11}); ^1H NMR, Tables 2 and 3; ^{13}C NMR, Table 4.

2.4. 3β-tert-Butyldimethylsilyloxy-5α,14α-cholestan-15-one (6a)

To a solution of **5a** (250 mg, 0.48 mmol) in CH_2Cl_2 (5 ml) was added sodium acetate (15 mg) and the mixture was cooled to 2–3°C in an ice bath. Pyridinium chlorochromate (166 mg; 0.77 mmol) was added and the mixture was stirred for 3 h under nitrogen. Upon completion of the reaction as judged by TLC, ether (25 ml) was added and the mixture was filtered through Florisil (10 g), followed by further elution with ether (50 ml). Evaporation of the filtrate gave a foamy solid (228 mg) that was subjected to MPLC (460 mm × 10 mm i.d. column; 20 g silica gel, 230–400 mesh; elution with 0.5% ethyl acetate in hexane). Evaporation of fractions 23–31 gave **6a** (216 mg; 87% yield), showing >99% purity by NMR: mp 208–209°C; TLC, single component in SS-2 (R_f 0.86) and SS-6 (R_f 0.89); $[\alpha]_D^{23}$ +33.8° (*c* 0.6, $CHCl_3$); IR ν_{max} 2955, 2930, 2857, 1728, 1468, 1385, 1254, 1134, 1105, 1094, 868, 835, 774 cm^{-1}; high resolution MS, *m/z* 516.4339 (M^+; calcd. for $C_{33}H_{60}O_2Si$, 516.4363), 459.3675 (100, $M-C_4H_9$), 107.0862 (5, C_8H_{11}); ^1H NMR, Tables 2 and 3; ^{13}C NMR, Table 4.

2.5. 3β-Hydroxy-5α,14α-cholestan-15-one (2a)

To a solution of TBDMS ether **6a** (100 mg, 0.19 mmol) in acetone (25 ml) was added *p*-toluenesulfonic acid (100 mg) and the mixture was stirred under nitrogen for 3 h. After TLC indicated that the reaction was complete, the mixture was diluted with water and extracted with ethyl acetate (3 × 25 ml). The organic phase was washed with 5% $NaHCO_3$ (2 × 25 ml) and water (3 × 150 ml), dried over Na_2SO_4 and evaporated to a solid (90 mg) that was subjected to MPLC (460 mm × 10 mm i.d. column; 20 g silica gel, 230–400 mesh; elution with 8% ethyl acetate in hexane). Evaporation of fractions 56–73 gave **2a** (69 mg; 89% yield), showing ≥99% purity by NMR: mp 175–176°C (lit. mp 174–175°C [18]); TLC, HPLC, and GC, Table 1; $[\alpha]_D^{23}$ +45.5° (*c* 0.6, $CHCl_3$), lit. $[\alpha]_D$ +47° [18]; IR ν_{max} 3300, 2928, 2855, 1728, 1467, 1450, 1383, 1076, 1043, 644 cm^{-1}; GC-MS, Table 1; ^1H NMR, Tables 2 and 3; ^{13}C NMR, Table 4.

2.6. 3β-tert-Butyldimethylsilyloxy-5α,14β-cholestan-15-one (6b)

Oxidation of **5b** (500 mg; 0.965 mmol) with pyridinium chlorochromate (332 mg; 1.54 mmol) in CH_2Cl_2 (10 ml) containing sodium acetate (30 mg) as described for the conversion of **5a** to **6a** gave a foamy solid (479 mg) that was subjected to MPLC (460 mm × 10 mm i.d. column; 20 g silica gel, 230–400 mesh; elution with 0.8% ethyl acetate in hexane). Evaporation of fractions 22–28 gave **6b** (455 mg; 91% yield), showing >99% purity by NMR: mp 100–101°C; TLC, single component in SS-2 (R_f 0.91) and SS-6 (R_f 0.94); $[\alpha]_D^{23}$ +10.8° (*c* 0.8, $CHCl_3$); IR ν_{max} 2957, 2931, 2859, 1736, 1462, 1383, 1370, 1252, 1103, 1084, 872, 835, 774 cm^{-1}; high resolution MS, *m/z* 501.4127 ($M-CH_3$, calcd. for $C_{32}H_{57}O_2Si$, 501.4128), 459.3687 (100, $M-C_4H_9$), 367.3379 (3, $C_{27}H_{43}$), 209.1905 (2, $C_{14}H_{25}O$, ring D + SC), 107.0857 (4, C_8H_{11}); ^1H NMR, Tables 2 and 3; ^{13}C NMR, Table 4.

2.7. Attempts to desilylate 6b to 2b

Desilylation of **6b** (50 mg; 0.1 mmol) by stirring overnight at room temperature with tetrabutylammonium fluoride (0.9 mmol in 1 ml of tetrahydrofuran) or with *p*-toluenesulfonic acid (50 mg in 2 ml of acetone) gave a ~5:1 mixture of **2b**:**2a** by ^1H NMR analysis.

2.8. 5α,14β-Cholestane-3β,15β-diol (4b)

Hydrolysis of TBDMS ether **5b** (700 mg, 1.32 mmol) for 8 h in acetone (50 ml) containing *p*-toluenesulfonic acid (700 mg) as described for the conversion of **6a** to **2a** gave a solid (545 mg) that was subjected to MPLC (460 mm × 10 mm i.d. column; 20 g silica gel, 230–400 mesh; elution with 20% ethyl acetate in hexane). Evaporation of fractions 67–108 gave **4b** (459 mg; 84% yield), showing ≥99% purity by NMR: mp 165.5–166.5°C lit. mp 166–167°C [16]; TLC, single component in SS-5 (R_f 0.47) and SS-8 (R_f 0.31); $[\alpha]_D^{23}$ +34.7° (*c* 0.6,

$CHCl_3$), lit. $[\alpha]_D$ +28.9° [16]; IR ν_{max} 3350, 2932, 2866, 1466, 1452, 1381, 1366, 1049, 1036 cm^{-1}; ^1H NMR, Tables 2 and 3; ^{13}C NMR, Table 4.

2.9. 3β-Hydroxy-5α,14β-cholestan-15-one (2b) and 5α,14β-cholestane-3,15-dione (7b)

Oxidation of diol **4b** (450 mg, 1.11 mmol) for 2.5 h in CH_2Cl_2 (50 ml) containing sodium acetate (40 mg) and pyridinium chlorochromate (383 mg; 1.77 mmol) as described for the conversion of **5a** to **6a** gave a foamy solid (430 mg) that was subjected to MPLC (960 mm × 10 mm i.d. column; 38 g silica gel, 230–400 mesh; elution with 9% ethyl acetate in hexane). Fractions 24–32 gave **7b** (227 mg; 51% yield), showing ~99% purity by NMR: mp 127.5–128.5°C; TLC, single component in SS-3 (R_f 0.77) and SS-7 (R_f 0.67); $[\alpha]_D^{23}$ +31.9° (*c* 0.8, $CHCl_3$); IR ν_{max} 2963, 2949, 2924, 2868, 1732, 1709, 1468, 1451, 1381, 1269, 1186, 1157, 1047, 995 cm^{-1}; ^1H NMR, Tables 2 and 3; ^{13}C NMR, Table 4.

Fractions 77–108 contained a mixture (189 mg) of **2b** and 15β-hydroxy-5α,14β-cholestan-3-one (**8**). The mixture was adsorbed onto 1 g of C_{18} silica gel and subjected to reverse phase MPLC on a Lobar RP-18 column (310 mm × 25 mm i.d.; elution with methanol). Fractions 5–6 gave **8** (8 mg), fractions 7–10 contained mixtures, and fractions 11–13 gave **2b** (72 mg) as a foamy solid: mp 65–66°C; TLC, HPLC, and GC, Table 1; $[\alpha]_D^{23}$ +16.2° (*c* 0.5, $CHCl_3$); IR ν_{max} 3400, 2951, 2930, 2864, 1734, 1466, 1383, 1368, 1057, 1034 cm^{-1}; high resolution MS, 402.3505 (4, M^+; calcd. for $C_{27}H_{46}O_2$, 402.3498), 289.2172 (10, M–SC), 234.1949 (2), 216.1876 (5, $C_{16}H_{24}$), 209.1899 (100, $C_{14}H_{25}O$, ring D + SC), 107.0857 (37, C_8H_{11}); GC-MS, Table 1; ^1H NMR, Tables 2 and 3; ^{13}C NMR, Table 4.

2.10. Equilibration of epimers 2a and 2b

A solution of **2a** (2 mg) in 20% KOH in ethanol (1 ml) was heated overnight at 70°C. Neutralization with 5% HCl (10 ml) followed by extraction with ethyl acetate, drying over Na_2SO_4 and evaporation of solvent gave a yellow oil. ^1H NMR analysis showed a 80:20 mixture of **2a**:**2b**. A parallel reaction of **2b** with ethanolic KOH gave **2a**:**2b** in a 79:21 ratio, in addition to an unidentified sterol (~30% of total material) showing the following ^1H NMR signals: δ 2.57 (td, 11.7, 3.8 Hz), 1.17 (s), 0.96 (d, 6.9 Hz), 0.88 (s). This byproduct was not observed in the equilibration of **2a**.

A solution of **2a** (2 mg) in acetone (2 ml) containing *p*-toluenesulfonic acid (6 mg) was heated overnight at 40–45°C. The cooled reaction was diluted with ethyl acetate (25 ml), washed with 5% $NaHCO_3$ solution (10 ml) and water (3 × 25 ml), dried over Na_2SO_4 and evaporated to a colorless oil. ^1H NMR analysis showed a 84:16 ratio of **2a**:**2b**. NMR analysis of a parallel equilibration of **2b** showed a 53:47 ratio of **2a**:**2b**. A similar reaction of **2a** (2 mg) with *p*-toluenesulfonic acid (5 mg) in acetone (1 ml) heated overnight at 60°C showed a 85:15 ratio of **2a**:**2b**. The parallel reaction of **2b** showed a 73:27 ratio of **2a**:**2b**.

2.11. 3β-Hydroxy-5α,14α-cholestan-15-one (2a) from 3β-hydroxy-5α-cholest-8(14)-en-15-one (1)

Freshly cut lithium pieces (150 mg, 22 mmol) were added to undistilled liquid ammonia (100 ml) cooled in a dry ice-acetone bath. After the lithium had dissolved (3–5 min), a cold solution of **1** (1 g) in anhydrous ether (50 ml) was added rapidly to the well-stirred solution and the mixture was stirred under nitrogen for 5 min. The reaction was quenched by addition of solid ammonium chloride (2.5 g) and the resulting colorless solution was immediately poured onto ice and extracted with ether (3 × 100 ml). The combined ether extracts were washed with water (3 × 250 ml) and dried over Na_2SO_4. Evaporation gave a white solid (1.1 g) that was adsorbed onto silica gel (4 g) and subjected to MPLC on a Lobar silica gel column (310 mm × 25 mm i.d.; elution with hexane (100 ml) and 15% ethyl acetate in hexane). Evaporation of fractions 47–69 gave a white solid (865 mg; 86% yield): mp, 175–176°C; single component by TLC in SS-4 (0.63) and SS-8 (0.49); ^1H NMR showed >99% purity and signals identical with those of authentic **2a**. In several repetitions of this procedure, **2a** was obtained together with variable amounts (up to 50%) of an unidentified byproduct showing distinctive NMR signals at δ_H 2.79 (dd, 18.6, 9.7 Hz), 2.55 (dq, 12, 3 Hz), 2.28 (br q, ~9.6

Hz), 0.907 (d, 6.5 Hz), 0.829 (s), 0.795 (s); δ_C 210.2, 94.9, 47.1, 47.0, 46.8, 19.9, 15.0.

The foregoing procedure was repeated except that the reaction was quenched by rapid addition of neat bromobenzene (2 ml). Evaporation of fractions 53–76 gave a white solid (960 mg; 96% yield): mp, 174–175°C; single component by TLC in SS-4 (0.63) and SS-8 (0.49); ^{1}H NMR showed >99% purity and signals identical with those of authentic **2a**. Similar lithium-ammonia reduction of **1** or its acetate ester with a *tert*-butanol quench gave a 2:1 ratio of **2a**:**4a**.

2.12. 3β-Hydroxy-5α,14α-cholestan-15-one (2a) from 5α,14α-cholestane-3,15-dione (7a)

To a solution of **7a** (100 mg; 0.25 mmol) in dry tetrahydrofuran (10 ml) was added lithium tri-*tert*-butoxyaluminum hydride (250 mg). After stirring for 1.5 min at 25°C under nitrogen, ice was cautiously added to decompose the excess hydride. The resulting mixture was poured into saturated NH_4Cl solution and thoroughly extracted with ether-CH_2Cl_2 (9:1). The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to a residue that was subjected to chromatography on a silica gel column (elution with 10% ethyl acetate in toluene). Evaporation of fractions containing the major product (as monitored by TLC with SS-9) followed by recrystallization of the residue from acetone-water gave **2a** (90 mg; 90% yield). A similar reaction using **7a** (1.9 g), tetrahydrofuran (190 ml) and lithium tri-*tert*-butoxyaluminum hydride (4.75 g) gave **2a** (1.72 g; 90% yield). A sample of **2a** prepared by this procedure was characterized as follows: mp, 174–175°C; single component by TLC in SS-4 (R_f 0.63) and SS-8 (R_f 0.49); ^{1}H NMR showed ≥99% purity and signals identical with those of authentic **2a**.

2.13. Effects of 3β-hydroxy-5α,14α-cholestan-15-one (2a) and 3β-hydroxy-5α,14β-cholestan-15-one (2b) on HMG-CoA reductase activity in CHO-K1 cells

Table 5 presents a comparison of the effects of the two saturated 15-ketosterols **2a** and **2b** on the levels of HMG-CoA reductase activity along with comparable data for the parent $\Delta^{8(14)}$-15-ketosterol **1**. Both **2a** and **2b** lowered the levels of reductase activity, with **2a** showing higher potency than **2b**. The potency of **2a** appeared to be slightly less than that of **1**.

3. Discussion

We initially prepared 3β-hydroxy-5α,14α-cholestan-15-one (**2a**) by selective reduction of 5α-cholestane-3,15-dione with lithium tri-*tert*-butoxyaluminum hydride. Although this reaction proceeded in 90% yield, we sought a synthesis from a more readily available starting material, such as the $\Delta^{8(14)}$-15-ketosterol **1** [15]. Saturated 15-ketosterols of the 14α configuration have been prepared by lithium-ammonia reduction of the corresponding $\Delta^{8(14)}$-15-ketosterol [18,19]. In

Table 5
Effect of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**1**), 3β-hydroxy-5α,14α-cholestan-15-one (**2a**) and its 14β epimer **2b** on the levels of HMG-CoA reductase activity in CHO-K1 cells

Sterol concentration (μM)	HMG-CoA reductase activity (% of control)		
	1[a]	**2a**[b]	**2b**[b]
0	100	100	100
0.1	62.5 ± 2.8	80.4 ± 4.9	92.6 ± 16.1
0.25	45.8 ± 2.0	47.5 ± 5.0	54.3 ± 12.1
0.5	36.6 ± 1.6	46.1 ± 11.4	51.2 ± 13.4
1.0	28.8 ± 1.4	39.0 ± 6.0	59.6 ± 15.3
2.5	23.6 ± 1.6	32.1 ± 4.9	49.7 ± 9.9

[a]Mean ± SEM of 40 independent experiments in which triplicate determinations of enzyme activity were made at each concentration.
[b]Mean ± SEM of 3 independent experiments in which triplicate determinations of enzyme activity were made at each concentration.

these procedures, the reaction was quenched with either *tert*-butanol [18] or ammonium chloride [19]. Quenching with *tert*-butanol generally leads to concomitant reduction of the keto group as a major byproduct [20,21]. Quenching with ammonium chloride is more common [21] but often gives variable results [20]. In our hands, lithium-ammonia reduction of **1** with an ammonium chloride quench usually gave modest yields of **2a** together with an unidentified byproduct, although an 86% yield of **2a** was obtained in one such reaction. By contrast, reductions quenched with bromobenzene [20] gave consistently high yields (e.g. 96%) of **2a** from **1**. The lithium-ammonia reductions produced negligible (~0.5%) amounts of the 14β epimer **2b**. The two syntheses giving consistently high yields of **2a** are shown in Fig. 3.

Preparation of the saturated 15-ketosterol of 14β configuration in high purity was far more challenging because of the difficulty of separating **2b** from **2a**. Although the C-14 epimers of 3-deoxy-15-ketosterols [22,23] and 3-deoxy-15-ketosteroids having short or absent side chains [23,24] can generally be separated by chromatography on silica gel, the 3-hydroxy-15-ketosterols **2a** and **2b** proved to be inseparable on silica gel (Table 1). Therefore, epimerization followed by chromatographic separation, a method used to prepare many saturated 15-ketosteroids with short side chains [23,24], could not be used to prepare **2b**. Whereas the 14α epimer could easily be isolated from epimeric mixtures by recrystallization, fractional recrystallization appeared to be incapable of producing **2b** of high purity, although the mother liquors may be enriched in **2b**. Another factor complicating the preparation of **2b** is the greater thermodynamic stability of the 14α epimer **2a**. Whereas the 14β epimers are more stable among 15-ketosteroids having a side chain smaller than ethyl [23], the 14α epimer is more stable among 15-ketosterols. For example, when 5α,14α-ergostan-15-one [23] or 5α,14β-cholestan-15-one [22] is subjected to conditions equilibrating the C-14 epimers, a 4:1 ratio favoring the 14α epimer is obtained. Because the equilibrium concentration of the 14β isomer **2b** is only ~20% (see below), **2b** becomes contaminated more readily than **2a** under epimerizing conditions.

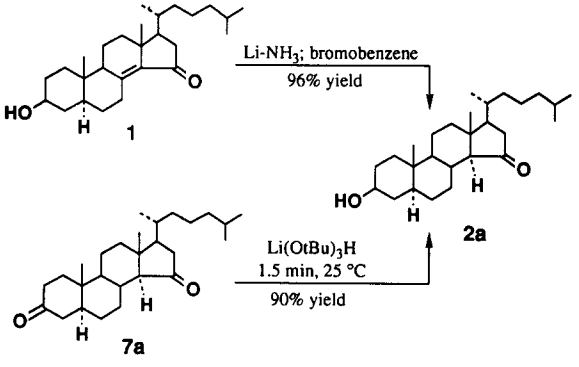

Fig. 3. Chemical synthesis of 3β-hydroxy-5α,14α-cholestan-15-one (**2a**) from 3β-hydroxy-5α-cholest-8(14)-en-15-one (**1**) or 5α-cholest-8(14)-ene-3,15-dione (**7a**).

A possible route to the 14β epimer **2b** was suggested by earlier work in which saturated 15-ketosterols were prepared by oxidation of saturated 15-hydroxysterols derived from isomerization-hydroboration of 5α-cholest-8(14)-en-3β-ol [16] or 5α-cholest-8-ene [22]. Following our previous work [16], we obtained a 22:78 mixture of saturated 15-hydroxysterols **4a** and **4b** by hydroboration-oxidation of $\Delta^{8(14)}$ acetate **3**. Diols **4a** and **4b**, which were inseparable on silica gel, were isolated by MPLC as their 3β-TBDMS ethers **5a** and **5b** (15% and 52% yield from **3**) and oxidized to the 15-ketosterol TBDMS ethers **6a** and **6b**. However, removal of the TBDMS group presented unanticipated difficulties. Although tetrabutylammonium fluoride converted **6a** cleanly to **2a**, treatment of **6b** with tetrabutylammonium fluoride or *p*-toluenesulfonic acid resulted in **2b** contaminated with 15-20% of the 14α-epimer **2a**. We then resorted to selective oxidation of diol **4b**, which was obtained by desilylation of **5b** with tetrabutylammonium fluoride. Oxidation of **4b** with pyridinium chlorochromate gave 3,15-dione **7b** together with the desired 15-ketosterol **2b** and small amounts of 15β-hydroxy-5α,14β-cholestan-3-one (**8**). MPLC separation of the mixture on silica gel gave dione **7b** in 51% yield and impure **2b** containing **8**, a mixture that was separated by reversed phase MPLC. The successful isolation of **2b** represents the first synthesis of a saturated 3-hydroxy-15-ketosterol of 14β configuration.

The tendency of the saturated 15-ketosterols to

epimerize readily under acidic or basic conditions, a major complication in the foregoing synthesis of **2b**, was investigated further. The 15-ketosterols **2a** and **2b** were each equilibrated in ethanolic KOH, giving a ~4:1 ratio of **2a**:**2b** in both cases by NMR analysis. This ratio corresponds to a energy difference (ΔG) of 0.8 kcal/mol and is in good agreement with force-field calculations showing the 14α epimer to be 0.5 kcal/mol lower in energy. This result is also compatible with earlier findings on saturated 15-ketosterols based on equilibration of a single epimer [22,23]. In similar experiments, equilibration was incomplete when the 15-ketosterols were treated with dilute *p*-toluenesulfonic acid in acetone.

The facile epimerization of the saturated 15-ketosterols may lead to errors in assessment of purity. For example, samples of both **2a** and **2b** of high purity by NMR showed contamination by the C-14 epimer upon GC analysis (Table 1), a result attributable to epimerization on catalytic sites in or near the hot injector. The extent of epimerization was dependent on the injector temperature and varied between the two GC instruments used. In a previous study [16], the 3,15-diketone **7a** was reported as contaminated by ~13% of the 14β epimer **7b** based on GC analysis, whereas evaluation by ^{1}H NMR at 500 MHz in the present work showed < 1% of **7b**. Similarly, an earlier GC analysis of the 14β epimer **7b** showed ~23% of **7a** [16], but subsequent NMR analysis indicated only 1–2% of **7a**. The apparent success of others [22] in measuring the epimeric purity of saturated 3-deoxy-15-ketosterols by GC may be attributable to different operating conditions. Other chromatographic methods were also of limited value in assessing the purity of samples of the saturated 15-ketosterols. Epimers **2a** and **2b** were inseparable on TLC (Table 1), as were diketones **7a** and **7b**. Epimerization during liquid chromatography is a further possible complication that has been reported for a saturated 15-ketosteroid chromatographed on alumina [25]. Epimers **2a** and **2b** are well-resolved by reversed phase HPLC (Table 1), but reliable quantitation of minor epimeric impurities is difficult with UV detection owing to very low extinction coefficients of the saturated sterols at wavelengths ≥210 nm. Although chromatographic methods could likely be developed based on HPLC using aromatic derivatives or GC under optimal operating conditions, we found NMR analysis to be a simple and effective method for analyzing the saturated 15-ketosterols.

Signals corresponding to epimeric impurities in **2a** or **2b** are easily resolved by ^{13}C NMR (Table 4), but the more sensitive ^{1}H NMR experiment is preferable for detecting minor impurities. The chemical shifts and couplings for ^{1}H signals of the 15-ketosterols are shown in Tables 2 and 3. For detecting 14α epimeric impurities in 15-ketosterols of 14β configuration, the δ 2.7 region (the H-7β signal of the 14α isomers **2a** and **7a**) is resolved from signals of **2b** and **7b**, even at relatively low field strengths. At higher fields (>300 MHz), H-16α and methyl signals of the 14α epimers may also be adequately isolated for quantitation without recourse to resolution enhancement. Detection of 14β impurities in samples of 15-ketosterols of 14α configuration is somewhat difficult at lower fields, but some lines of the H-7α signal at δ ~2.5 (qd, 13.2, 4.5 Hz) are likely to be resolved. These observations should also be useful in determining the configuration of saturated 15-ketosteroids of unknown stereochemistry.

Full assignments are given for the ^{1}H and ^{13}C NMR spectra of the 14α- and 14β-epimers of the 15-oxygenated sterols described herein (Tables 2–4). These assignments, which are among the first extensive NMR assignments for any saturated 14β-sterols, were established definitively by NOE difference experiments in conjunction with other standard 1D and 2D NMR techniques [11]. The ^{13}C assignments are compatible with earlier assignments for 3-deoxy analogs of the 15α-hydroxysterol **4a** [13] and the 15-ketosterol **2a** [12]. The assignments allow calculation of substituent increments for introduction of a 15-hydroxy or 15-keto group, and the ^{1}H data provide the basis for designing and interpreting NOE difference experiments.

The NMR data in Tables 2–4 also furnished detailed information facilitating conformational analysis. In view of the twist form of ring C noted previously for a 14β-sterol [26] and the unusual high-field shift for H-12β of **2b** (δ 1.33), we in-

vestigated the conformation of ring C of the 14β-sterols described here. Use of ^1H-^1H coupling constants and NOE difference experiments to establish the conformation of ring C was hampered by severe overlap and strong coupling in the ^1H NMR spectra of the 14β-sterols. In the 14β-15β-hydroxysterols **4b** and **5b**, five different resonances, including H-11β, H-12α and H-12β are located in the δ 1.17–1.21 region. Strong coupling between H-11β and H-12α similarly complicated spectral analysis in the 14β-15-ketosterols **2b** and **6b**. However, effects of the 3-keto group of **7b** dispersed the cluster of signals at δ ~ 1.19 sufficiently so that both C-11 resonances could be identified with the aid of difference spectra. A NOE from H-19 to the upfield C-11 signal established this as H-11β and the couplings of this signal (roughly tdd, ~13.2, 12.0, 3.3 Hz) demonstrating its axial orientation showed that ring C is a chair. Also in support of the chair conformation for ring C was an NOE from H-11α to H-1β, the 2.1-Hz $J_{12\beta-14\beta}$ coupling suggesting planarity of the intervening atoms and force-field calculations showing twist forms of ring C to be ~2.7 kcal/mol higher in energy than the best chair conformation. Chair conformations for ring C of **2b** and **6b** were evident from chemical shift comparisons with **7b**.

The saturated 15-ketosterol functionality fortuitously led to an unusually favorable dispersion of the ^1H NMR signals of the C_8H_{17} sterol side chain, whose coupling patterns are normally inscrutable owing to strong coupling effects. The dispersion of signals for both C-14 epimers of the 15-ketosterols permitted the first analyses of C-24 proton couplings in a C_8H_{17} sterol side chain (Table 3). These results are compatible with a predominantly extended conformation of the side chain having slightly unequal populations of the minor C23–C24 rotamers and of the major C24–C25 rotamers. An unusual side-chain conformation for 15-ketosterols of 14β configuration can be inferred from earlier work [27,28]. A detailed conformational analysis of the side chain of **2b** will be described elsewhere.

The saturated 15-ketosterols were also characterized by mp, IR, optical rotation, MS, TLC, HPLC, and GC. The IR carbonyl absorbance of the 14β isomers were at slightly higher wave numbers (~1734 cm^{-1}) than those of the 14α isomers (1728 cm^{-1}). Although this difference might be used to assign the C-14 configuration of saturated 15-ketosteroids, it is too small to estimate levels of epimeric contamination. The MS of the saturated 15-ketosterols **2a** and **2b** (Table 1, footnote f) were markedly different, paralleling differences observed for the C-14 epimers of 5α-androstan-15-one [25]. The MS of **2b** and its TMS ether were dominated by a single ion at *m/z* 209, whose origin has been studied extensively among similar 15-ketosteroids [19,24,25,29,30]. This ion is observed in substantially lower intensity for the 14α epimer **2a**, whose MS shows several strong ions of comparable abundance, as described previously [29]. Mass spectra of the TBDMS ethers (**5a**, **5b**, **6a**, **6b**) were relatively uninformative, being dominated by a single ion corresponding to loss of the *tert*-butyl radical. The *m/z* 209 ion showed only 2% relative abundance in the high resolution MS of TBDMS ether **6b**, an illustration of a striking change in fragmentation pathways when the saturated 15-ketosterols are derivatized as TBDMS ethers.

The saturated 15-ketosterols **2a** and **2b** lowered the levels of HMG-CoA reductase activity in CHO-K1 cells. The 15-ketosterol **2a** with the natural *trans* C-D ring junction was more potent than its epimer **2b** with the *cis* C-D ring junction. **2a** appeared to be slightly less active than the parent $\Delta^{8(14)}$-15-ketosterol **1**. Thus, the $\Delta^{8(14)}$ double bond of **1** is not essential for lowering of reductase activity in these cells. This finding is of importance in considerations of the development of new analogs of the parent 15-ketosterol **1**. Studies of the metabolism of **1** have indicated conversion to cholesterol [31–39] and to polar metabolites which appear to arise largely by oxidation of the side chain of **1** [36,38,40,41]. The results of recent studies have demonstrated that 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-15-one [9], in which conversion of **1** to its major side-chain oxygenated metabolites is blocked, undergoes substantial metabolism to 25,26,26,26,27,27,27-heptafluorocholesterol [42], presumably by the same series of reactions involved in the overall conversion of **1** to cholesterol [32]. On the basis of knowledge of the reactions involved in this process

[32 and references cited therein], it can be predicted that **2a** would not undergo metabolism to cholesterol. Thus, the 25,26,26,26,27,27,27-heptafluoro analog of **2a** represents an analog of **1** in which neither its conversion to F_7-cholesterol nor its side-chain oxidation would be anticipated. Stimulated by the demonstration that **2a** is highly active in lowering HMG-COA reductase activity, we have pursued the preparation of its F_7 analog and, in preliminary experiments, have shown that it shows high activity in lowering reductase activity in cultured cells and lowers serum cholesterol levels upon dietary administration to rats [43].

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