Sterols affecting meiosis: novel chemical syntheses and the biological activity and spectral properties of the synthetic sterols

Benfang Ruan,* Shinya Watanabe,[1,†] John J. Eppig,[†] Christopher Kwoh,* Natasha Dzidic,* Jihai Pang,* William K. Wilson,* and George J. Schroepfer, Jr.[2,*]

Departments of Chemistry and of Biochemistry and Cell Biology,* Rice University, 6100 Main Street, Houston, TX 77251-1892; and the Jackson Laboratory,[†] Bar Harbor, ME 04609-1500

Abstract 4,4-Dimethyl-5α-cholesta-8,14,24-trien-3β-ol (I) from human follicular fluid and 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol (II) from bull testes have been reported to activate meiosis in mouse oocytes (Byskov et al., 1995. *Nature.* 374: 559–562). Described herein are new chemical syntheses of I, II, and the $\Delta^{8(14),24}$ analog XXII. A critical step in these syntheses was a remarkably high yield side chain oxidation of 3β-acetoxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one to the corresponding C_{24} 24-hydroxy compound VI. Oxidation of VI to the aldehyde, followed by Wittig olefination gave 3β-acetoxy-4,4-dimethyl-5α-cholesta-8(14),24-dien-15-one. Reduction with sodium borohydride to the 15β-hydroxysteryl ester, dehydration with sulfuric acid in $CHCl_3$, and saponification furnished I in high purity. Reduction of VI with sodium borohydride to the 15-hydroxysteroid followed by dehydration gave 3β-acetoxy-4,4-dimethyl-5α-chola-8,14-dien-24-ol. Hydrogenation over Raney nickel gave the monounsaturated $\Delta^{8(14)}$ and Δ^{8} compounds. Oxidation to the corresponding aldehydes followed by Wittig olefination and saponification gave II and XXII. Chromatographic, mass spectral, and ^{1}H and ^{13}C nuclear magnetic resonance spectral data have been presented for the synthetic sterols and their derivatives. I, II, XXII, and their $\Delta^{8,14}$ and $\Delta^{7,14}$ analogs, at 3 μg per ml, caused a resumption of meiosis in mouse oocytes in the presence of hypoxanthine (3.5 mM). Under the same conditions, Δ^{5} and $\Delta^{5,7}$ sterols were inactive.

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4,4-Dimethyl-5α-cholesta-8,14,24-trien-3β-ol (**I**), 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol (**II**), and 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol (**III**) (**Fig. 1**) have recently been reported to activate meiosis in mammalian oocytes (1). **I** and **II** were samples isolated from human follicular fluid and bull testicular tissue, respectively, with chemical characterization based on gas chromatography–mass spectrometry (GC–MS) and, in the case of **II**, ^{13}C nuclear magnetic resonance (NMR) spectroscopy in the absence of authentic standards. The $\Delta^{8,14,24}$-triene **I** and the $\Delta^{8,14}$-diene **III** are products of a cytochrome P-450-dependent 14α-demethylation of lanosterol and 24,25-dihydrolanosterol, respectively (2–7). **I**, **II**, and **III** represent potential intermediates in the biosynthesis of cholesterol (8). The enzymatic formation of **I** from lanosterol in rat ovary has been recently reported to be under hormonal control (9), and **I** has also been reported to be a positive regulator of the nuclear orphan receptor LXR_{α} (10).

Described herein are new approaches to the chemical syntheses of **I** (**Fig. 2**) and **II** (**Fig. 3**). Also presented are chromatographic, mass spectral (MS), and ^{1}H and ^{13}C NMR spectral properties of **I**, **II**, and **III**, their derivatives, and related compounds, as well as the results demonstrating the ability of the synthetic sterols to affect meiosis in mouse oocytes. Preliminary communications of portions of this work have been presented (11, 12).

EXPERIMENTAL PROCEDURES AND RESULTS

General methods and materials

Melting points (MP) were measured in sealed, evacuated capillary tubes using a Thomas-Hoover apparatus. Ul-

Abbreviations: Ag^{+}, silver ion; GC, gas chromatography; GVB, germinal vesicle breakdown; HPLC, high performance liquid chromatography; IR, infrared; MP, melting point; MPLC, medium pressure liquid chromatography; MS, mass spectra or mass spectrometry; MTBE, methyl *tert*-butyl ether; NMR, nuclear magnetic resonance spectroscopy; RRT, relative retention time; THF, tetrahydrofuran; TLC, thin layer chromatography; UV, ultraviolet.

[1]Current address: Kyushu National Agricultural Experiment Station, 2421 Nishigushi, Kikuchi, Kumamoto 861-11, Japan.

[2]To whom correspondence should be addressed.

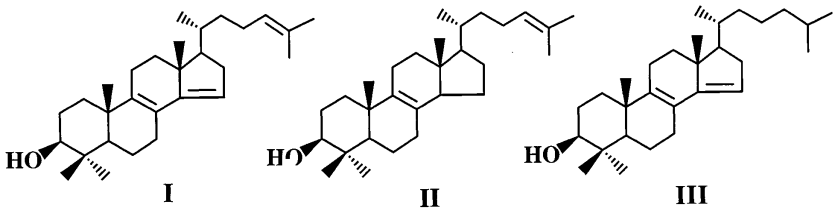

Fig. 1. Structure of sterols reported by Byskov et al. (1) to activate meiosis.

traviolet (UV) spectra were recorded on a Shimadzu UV-1601 spectrophotometer using ethanol as solvent. Infrared (IR) spectra were recorded on a Mattson Galaxy 6020 Fourier-transform IR spectrometer with KBr pellets. Low resolution and high resolution MS were recorded on a VG ZAB-HF double sector instrument with an electron energy of 70 eV and direct inlet sample introduction. The composition and origins of ion B, ion B-CH_3, and ion D in the mass spectra of C_{24} (13, 14) and C_{27} (15–19) $\Delta^{8(14)}$-15-ketosteroids have been presented previously. Ions designated with an asterisk (*) showed exact mass values on high resolution MS which were within 3 millimass units of the calculated values for the proposed ions. GC–MS was carried out on the same instrument coupled to an HP-5890-A GC unit using a 60 m DB-5MS column (0.25 mm ID; 0.1 μm film thickness; J&W Scientific Inc., Folsom, CA). The column temperature was 250°C and the temperatures of the injector and GC–MS interface were 290°C. Helium was used as the carrier gas with a head pressure of 20 psi. Trimethylsilyl ether derivatives were prepared by treatment of the steroids with a 1:1 mixture of bis(trimethylsilyl)trifluoroacetamide and pyridine for 2 h under nitrogen at 40°C, followed by evaporation to dryness at 40°C under nitrogen. NMR spectra were recorded on a Bruker AMX500 (500.1 MHz, 25°C for ^{1}H, 22°C for ^{13}C) spectrometer in $CDCl_3$ solution (5–20 mm for ^{1}H; 20–100 mm for ^{13}C) and referenced to an internal standard of tetramethylsilane (^{1}H) or $CDCl_3$ at 77.0 ppm (^{13}C).

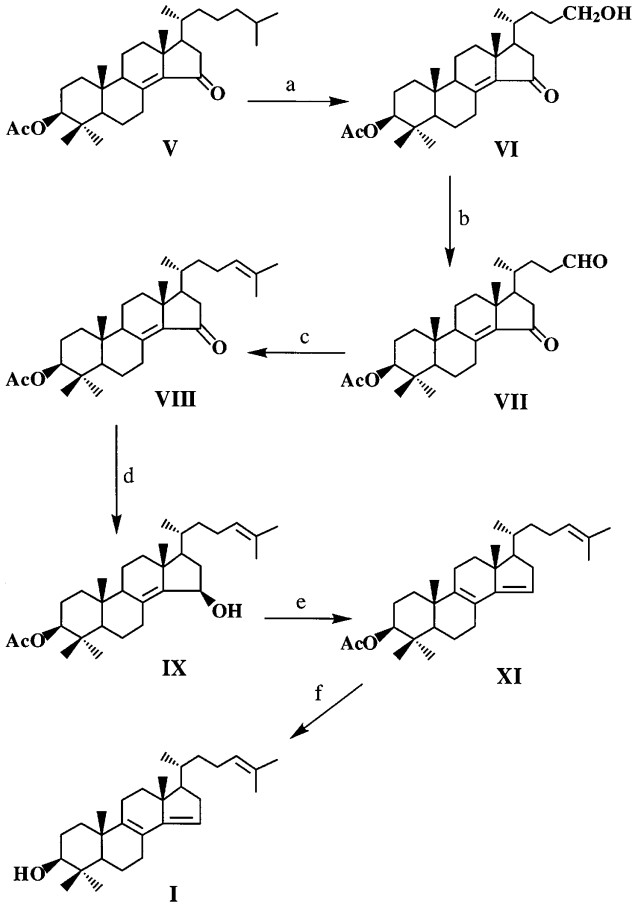

Fig. 2. Conversion of 3β-acetoxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one (**V**) to 4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol (**I**): (a) $(CF_3CO)_2O$, H_2O_2, H_2SO_4, −2°C; Na_2SO_3, K_2CO_3; (b) oxalyl chloride, dimethylsulfoxide, CH_2Cl_2, −50°C, 1 h; triethylamine, 25°C, 5 min; (c) isopropyltriphenylphosphonium iodide, butyllithium, tetrahydrofuran, 0°C, 2 h; (d) $NaBH_4$, ethanol, 25°C; (e) H_2SO_4 (cat.), $CHCl_3$, 25°C, 5 min; (f) KOH, ethanol, 70°C, 2 h.

Thin-layer chromatography (TLC) was carried out using aluminum-backed silica gel 60 plates (EM Science, Gibbstown, NJ). Components on the plates were visualized after spraying with 5% ammonium molybdate in 10% sulfuric acid and heating. Unless specified otherwise, medium pressure liquid chromatography (MPLC) was carried out on glass columns packed with silica gel (230–400 mesh), whereas silica gel (70–230 mesh) was used for routine column chromatography.

High performance liquid chromatography (HPLC) was carried out using a Rheodyne 7125 or Waters U6K injector and Waters 600 or 510 pumps with UV detection at 210 nm. Reversed phase HPLC was carried out using a Customsil ODS column (250 mm × 4.6 mm i.d.; Custom LC, Houston, TX) using methanol as solvent at a flow rate of 1 ml per min. Ag^+-HPLC was carried out as described previously (20). In the current study 5 μm Nucleosil SA cation exchange (100 Å pore size) columns (250 mm × 4.6 mm, 300 mm × 3.2 mm, 300 mm × 4.6 mm, and 250 mm × 10 mm) were obtained from Alltech Associates (Deerfield, IL), and they were charged with silver ion and prepared for use as described previously (20). Flow rates for analytical and semipreparative (10 mm i.d.) columns were 1 ml per min and 3 ml per min, respectively.

4,4-Dimethylcholest-5-en-3β-ol, 4,4-dimethylcholesta-5,7-dien-3β-ol, 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol (**III**), and 4,4-dimethyl-5α-cholesta-7,14-dien-3β-ol (**IV**) were prepared as described previously (21) and, after chromatographic purification, showed purities of >99%, ~97%, ~95%, and ~96%, respectively, as judged by capillary GC and ^{1}H NMR. (20R,22R)-cholest-5-ene-3β,20,22-triol and (20S,22S)-cholest-5-ene-3β,20,22-triol were prepared as described previously (22), and each showed a purity >99% on the basis of TLC, Ag^+-HPLC, and ^{1}H NMR. Samples of (22R)-cholest-5-ene-

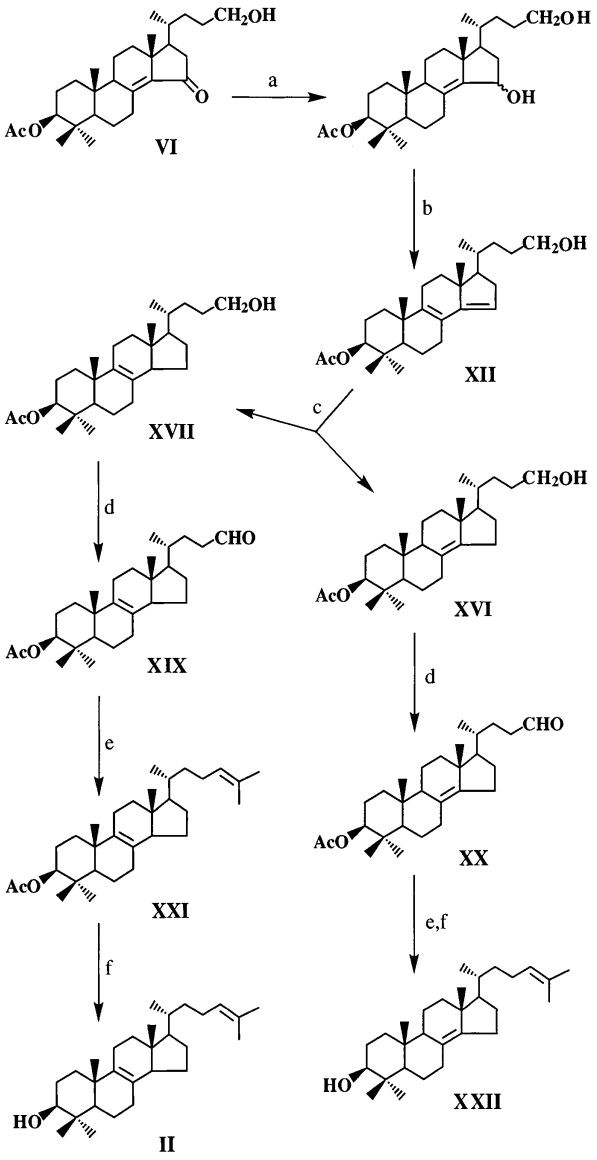

Fig. 3. Synthesis of 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol (**II**) and 4,4-dimethyl-5α-cholesta-8(14),24-dien-3β-ol (**XXII**). (a) $NaBH_4$, ethanol, 25°C; (b) H_2SO_4 (cat.), $CHCl_3$, 25°C, 15 min; (c) hydrogenation, Raney nickel; (d) oxalyl chloride, dimethyl sulfoxide, CH_2Cl_2, −50°C, 1 h; triethylamine, 25°C, 5 min; (e) isopropyltriphenylphosphonium iodide, butyllithium, tetrahydrofuran, 0°C, 2 h; (f) KOH, 95% ethanol, 60°C, 2 h.

3β,22-diol and (22S)-cholest-5-ene-3β,22-diol, showing minor impurities on TLC (overloaded), were purchased from Sigma Chemical Company (St. Louis, MO). Steryl acetates were prepared by treatment of the free sterols with a mixture of acetic anhydride and pyridine (1:1). Bovine serum albumin (crystallized) was obtained from ICN Biochemical (Aurora, OH) and equine chorionic gonadotropin was from Dyosynth (Oss, Holland).

Assays of activation of meiosis

Oocyte-cumulus cell complexes were isolated from the ovaries of 22–24 day old B6SJLF1 mice 44 h after intraperitoneal injection of equine chorionic gonadotropin (5 IU). The culture medium was minimum essential medium with Earle's salts supplemented with crystallized bovine serum albumin (5 mg/ml). The medium also contained hypoxanthine (3.5 mm) to suppress the spontaneous resumption of meiosis (23). Cumulus cells were removed from oocytes by drawing the complexes in and out of a small-bore glass pipet. The denuded oocytes were incubated in 2.5 ml of medium with or without sterols for 15–17 h at 37°C in an atmosphere of 5% O_2, 5% CO_2, and 90% N_2. Sterols were dissolved in ethanol at a concentration of 1 mg/ml. All sterols were tested at a concentration of 3 μg/ml. Control cultures received the same amount of ethanol as the sterol-treated groups. After the incubation period, the percentage of oocytes that underwent germinal vesicle breakdown, indicative of the resumption of meiosis, was determined. Approximately 40 cumulus cell-free oocytes were distributed to each group. Data are presented as the mean percentage of four independent experimental replicates; variation among experiments is illustrated using the standard error of the mean. For evaluation of the differences among groups, data were subjected to arcsin transformation and ANOVA. When a significant F-ratio was defined by ANOVA, groups were compared using the Fisher's PLSD post-hoc test using software; when $P \leq 0.05$, the difference was considered significant.

Syntheses of 4,4-dimethylsterols

*3β-Acetoxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one (**V**).* 3β-Acetoxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one was prepared by treatment of 3β-hydroxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one, obtained by minor modifications of a previously described synthesis (21), with acetic anhydride and pyridine. The crude product was subjected to MPLC on a silica gel column (50 cm × 2.5 cm) using 2% ethyl acetate in hexane as the eluting solvent. Fractions (20 ml) were collected every 4.5 min. The contents of fractions 159–235 were combined and evaporated to dryness to give **V** as a white solid; MP 123.5–126°C; single component (R_f 0.9) on TLC (solvent, 25% ethyl acetate in hexane) and a single component (t_R 9.8 min) on reversed phase HPLC; MS, 470 (100%; M^+), 452* (7%, M–H_2O), 410* (20%; M–CH_3COOH), 395* (31%; M–CH_3COOH–CH_3), 367 (11%; $C_{26}H_{39}O$), 357* (3%; M–SC), 339* (17%, M–SC–H_2O), 297* (11%; M–CH_3COOH–SC), 276* (21%; ion B), 261* (22%; ion B–CH_3), and 135* (91%; $C_{10}H_{15}$); high resolution MS, calcd. for $C_{31}H_{50}O_3$ 470.3760, found 470.3760; IR, ν_{max} 2963, 2872, 2853, 1732, 1703, 1626, 1466, 1365, 1240, 1084, and 1026 cm^{-1}; UV, λ_{max} 259 nm (ε 14,000); ^1H NMR, **Table 1**; ^{13}C NMR, **Table 2**.

*3β-Acetoxy-24-hydroxy-4,4-dimethyl-5α-chol-8(14)-en-15-one (**VI**).* To a mechanically stirred mixture of trifluoroacetic anhydride (25 ml) and sulfuric acid (10 ml) maintained at −10°C was added a solution of 30% hydrogen peroxide (2.5 ml) dropwise over a period of 30 min. During the addition the temperature of the mixture was maintained at between −4°C and −8°C. Acetate **V** (1.00 g; 2.13 mmol) was, with continued vigorous stirring, added in one portion. The color of the reaction turned to orange and the temperature of the reaction mixture was increased to –2°C. Within 1 h, the mixture, maintained at −2°C, turned to a

TABLE 1. ^{1}H NMR chemical shifts for 3β-acetoxy-4,4-dimethyl-$\Delta^{8(14)}$-15-oxygenated steroids and unsaturated 3β-acetoxy-4,4-dimethyl-C_{24}-steroids[a,b,c]

	4,4-Dimethyl-$\Delta^{8(14)}$-15-oxygenated Steroids						4,4-Dimethyl Unsaturated Steroids								
	15-one **V**	15-one 24-ol **VI**	15-one 24-al **VII**	15-one Δ^{24} **VIII**	15β-ol Δ^{24} IX	15α-ol Δ^{24} **X**	$\Delta^{8,14}$ 24-ol **XII**	$\Delta^{7,14}$ 24-ol **XIII**	$\Delta^{6,8(14)}$ 24-ol **XIV**	$\Delta^{8(14),15}$ 24-ol **XV**	$\Delta^{8(14)}$ 24-ol **XVI**	Δ^{8} 24-ol **XVII**	Δ^{7} 24-ol **XVIII**	Δ^{8} 24-al **XIX**	$\Delta^{8(14)}$ 24-al **XX**
H–1α	1.333	1.333	1.333	1.333	1.267	1.269	1.390	1.249	1.255	1.261	1.241	1.340	1.263	1.340	1.242
H–1β	1.693	1.693	1.690	1.693	1.686†	1.680	1.844	1.847	1.659	1.677	1.669†	1.746	1.824	1.743	1.667
H–2α	1.714	1.715†	1.717†	1.714†	1.692†	1.693†	1.762†	1.669†	1.757†	1.689†	1.672†	1.715†	1.645†	1.716†	1.674†
H–2β	1.598	1.597	1.597	1.598	1.600†	1.594	1.654†	1.628†	1.676†	1.600†	1.588†	1.623†	1.609†	1.623†	1.589†
H–3α	4.539	4.539	4.539	4.539	4.536	4.535	4.489	4.501	4.559	4.536	4.529	4.494	4.513	4.493	4.528
H–5α	1.249	1.250	1.252	1.249	1.130	1.145	1.302	1.275	1.990	1.127	1.077	1.209	1.236	1.208	1.078
H–6α	1.745	1.747	1.751	1.745	1.720	1.659	1.751	2.04	5.587	1.623	1.602	1.664	1.97	1.666	1.607
H–6β	1.484†	1.481†	1.486†	1.485†	1.377	1.388	1.563	2.05		1.386	1.308	1.508	1.97	1.510	1.310
H–7α	1.557†	1.560†	1.562†	1.560†	1.772	1.967	2.309†	5.817	6.233	1.792	1.721	1.928†	5.206	1.930†	1.723
H–7β	4.184	4.183	4.184	4.181	2.808	2.750	2.188†			2.636	2.443	2.053†		2.051†	2.444
H–9α	1.860	1.865	1.868	1.861	1.674	1.784		1.753	1.921	1.753	1.629†		1.668†		1.633
H–11α	1.578†	1.584†	1.588†	1.580†	1.541†	1.562†	2.17	1.531†	1.545	1.550†	1.525†	2.05*	1.500†	2.05*	1.530
H–11β	1.490†	1.493†	1.492†	1.490†	1.459†	1.513†	2.17	1.461†	1.418	1.484†	1.440†	2.04*	1.433†	2.04*	1.439
H–12α	1.237	1.242	1.245	1.238	1.048	1.219	1.390	1.302	1.247	1.445†	1.110	1.376	1.215	1.382	1.114
H–12β	2.089	2.091	2.083	2.089	1.904	1.976	2.011	2.020	1.997	1.932	1.926	1.953	2.010	1.941	1.915
H–14α												2.029†	1.779	2.038†	
H–15α					4.632		5.359	5.512	2.386	5.899	2.183	1.585	1.542	1.603	2.199
H–15β						4.694			2.305		2.248	1.277†	1.396	1.290	2.263
H–16α	2.349	2.357	2.374	2.360	2.352	1.838	2.363	2.324	1.904	6.343	1.826	1.900	1.895	1.920	1.835
H–16β	2.048	2.060	2.094	2.052	1.418	1.673	2.068	1.932	1.447		1.377	1.321†	1.276†	1.350†	1.402
H–17α	1.455	1.471	1.468	1.465	1.070	1.485†	1.529	1.597†	1.192	2.086	1.128	1.154	1.223†	1.156	1.128
H–18	0.969	0.976	0.978	0.969	1.022	0.822	0.811	0.822	0.893	0.973	0.842	0.595	0.524	0.593	0.842
H–19	0.800	0.801	0.801	0.801	0.805	0.834	1.054	0.869	0.700	0.853	0.775	1.006	0.883	1.005	0.774
H–20	1.570	1.614	1.632	1.583	1.550	1.485†	1.651	1.630†	1.509†	1.663†	1.494†	1.422	1.404	1.449	1.526
H–21	0.994	1.020	1.004	1.013	0.942	0.966	0.959	0.948	0.963	0.972	0.951	0.944	0.943	0.934	0.941
H–22R	1.326†	1.458†	1.806	1.396	1.442	1.462†	1.488†	1.478†	1.495†	1.64§	1.492†	1.452†	1.455†	1.800	1.833
H–22S	1.068	1.143	1.369	1.105	1.092	1.133	1.126	1.120	1.144	1.236	1.145	1.063	1.082	1.318	1.400
H–23R	1.326†	1.478†	2.406	1.887	1.868	1.874	1.484†	1.478†	1.466†	1.509	1.463†	1.450†	1.454†	2.354	2.364
H–23S	1.181†	1.633	2.472	2.019	2.002	2.045	1.664	1.663	1.639	1.675	1.637	1.644	1.645	2.457	2.454
H–24R	1.097†	3.615*	9.776	5.072	5.089	5.091	3.63*	3.627*	3.61*	3.64*	3.61*	3.608*	3.602†	9.770	9.777
H–24S	1.146†	3.632*					3.65*	3.642*	3.64*	3.66*	3.63*	3.627*	3.635†		
H–25	1.512														
H–26	0.864			1.682	1.683	1.682									
H–27	0.862			1.596	1.599	1.603									
H–28	0.909	0.910	0.911	0.909	0.898	0.892*	0.898	0.869	0.943	0.891*	0.882	0.876	0.875	0.876	0.884
H–29	0.889	0.889	0.890	0.889	0.890	0.894*	0.903	0.967	0.896	0.898*	0.883	0.883	0.970	0.883	0.884
Ac	2.058	2.059	2.059	2.059	2.056	2.055	2.056	2.056	2.063	2.055	2.052	2.051	2.054	2.051	2.053

[a] Data obtained at 500 MHz in 3–15 mm $CDCl_3$ solution at 25°C and referenced to $Si(CH_3)_4$. Chemical shifts given to two (three) decimal places are generally accurate to ±0.01 (±0.001) ppm except that values marked by † are accurate to about ±0.003 ppm. R and S denote pro-*R* and pro-*S* hydrogens. Adjacent assignments marked with an asterisk may be interchanged.

[b] Data for **XIV** and **XV** are based only on 1D and COSYDEC spectra. Assignment marked by § is tentative, as is the structure of **XV**.

[c] Coupling constants were very similar to those expected based on data reported for similar steroids. Selected coupling constants for **XV**: H–3α, dd, 11.7, 4.1 Hz; H–5α, dd, 12.4, 2.6 Hz; H–6β, qd, 12.9, 4.5 Hz; H–7α, br td, 13.7, 5.2 Hz; H–7β, ddd, 14.4, 4.5, 2.3 Hz, H–15, ddt, 6.0, 1.9, 0.8 Hz; H–16, dd, 6.0, 2.9 Hz; H–17α, ddq, 10.5, 2.9, 1.3 Hz; H–18, s;H–19, d, 0.5 Hz; H–21, d, 6.6 Hz; H–28, s; H–29, s.

thick slurry. With continued vigorous stirring for 3 h, the slurry changed to a clear, light yellow-colored, mobile solution. TLC (solvent, 25% ethyl acetate in hexane) of an aliquot of the reaction mixture indicated completion of the reaction as judged by consumption of almost all of the starting material. The reaction mixture was poured into ice water (100 ml) containing sodium sulfite (200 mg). To the resulting mixture, potassium carbonate (~20 g) was slowly added and, after stirring for 30 min, the mixture was extracted 4 times with ethyl acetate (200 ml portions), and the combined organic extracts were washed 5 times with 10% KOH (80 ml portions), once with a saturated $NaHCO_3$ solution (50 ml), twice with brine (50 ml), and dried over anhydrous sodium sulfate. The crude product (1.4 g), obtained upon evaporation of the solvent under reduced pressure, showing a single component on TLC, was dissolved in $CHCl_3$ (2 ml) and applied to a silica gel column (25 cm × 2.5 cm). Using 5% acetone in hexane as the eluting solvent, fractions (20 ml) were collected every 3.5 min. The contents of fractions 37 to 47 were combined and, after evaporation of the solvent under reduced pressure, gave **VI** (690 mg; 73% yield), MP 218–220°C; single component (R_f 0.12) on TLC (solvent, 25% ethyl acetate in hexane) and a single component (t_R 3.82 min) on reversed phase HPLC, and (as its TMS ether derivative) it showed one major (~98%) component (t_R 48.12 min) on capillary GC analysis on a DB-5 column (60 m); GC–MS (TMS ether), 516 (100%; M^+), 501 (16%; M–CH_3), 498 (3%; M–H_2O), 456 (14%; M–CH_3COOH), 441 (16%; M–CH_3COOH–CH_3), 413 (6%; ion D), 339 (11%), 333 (4%), 322 (8%), 307 (7%), 305 (5%), 297 (7%), 279 (9%), 159 (11%), and 135 (62%); MS (free sterol), 444

TABLE 2. ^{13}C NMR chemical shifts for 3β-acetoxy-4,4-dimethyl-15-oxygenated steroids and unsaturated 3β-acetoxy-4,4-dimethyl-C_{24}-steroids[a]

	4,4-Dimethyl-$\Delta^{8(14)}$-15-oxygenated steroids					4,4-Dimethyl Unsaturated Steroids						
	15-one **V**	15-one 24-ol **VI**	15-one 24-al **VII**	15-one Δ^{24} **VIII**	15β-ol Δ^{24} **IX**	$\Delta^{8,14}$ 24-ol **XII**	$\Delta^{7,14}$ 24-ol **XIII**	$\Delta^{8(14)}$ 24-ol **XVI**	Δ^{8} 24-ol **XVII**	Δ^{7} 24-ol **XVIII**	Δ^{8} 24-al **XIX**	$\Delta^{8(14)}$ 24-al **XX**
C–1	36.63	36.63	36.62	36.63	36.61	35.67	37.29	36.67	35.37	37.67	35.37	36.67
C–2	23.90	23.89	23.88	23.90	24.03	24.19	23.97	24.11	24.18	23.97	24.17	24.10
C–3	80.68	80.68	80.63	80.68	80.91	80.66	81.06	81.12	80.92	81.18	80.88	81.09
C–4	37.84	37.84	37.83	37.84	37.83*	37.90	37.47	37.82	37.80	37.46	37.80	37.83
C–5	54.15	54.14	54.12	54.14	54.35	50.42	49.51	54.31	50.25	50.10	50.24	54.30
C–6	22.27	22.27	22.25	22.27	22.28	18.15	23.44	21.69	18.29	22.67	18.27	21.68
C–7	27.83	27.84	27.83	27.83	29.33	27.65	120.55	29.99	28.25	117.83	28.24	29.99
C–8	150.08	150.33	150.56	150.08	133.08	122.73	133.52	126.06	127.86	138.78	127.77	126.29
C–9	52.71	52.69	52.66	52.70	51.29	141.50	52.16	51.30	135.58	51.84	135.62	51.27
C–10	39.62	39.63	39.64	39.62	37.94*	37.60	34.89	37.60	36.82	35.29	36.81	37.61
C–11	19.08	19.07	19.01	19.08	19.31	21.10	20.43	19.43	22.01	20.97	21.99	19.41
C–12	36.96	36.96	36.93	36.94	37.40	36.92	39.85	37.26	36.85	39.30	36.80	37.24
C–13	42.47*	42.47	42.45	42.49	42.09	44.93	46.33	42.60	42.03	43.25	42.09	42.63
C–14	140.00	139.84	139.60	139.96	145.09	150.92	151.61	142.10	51.77	54.80	51.74	141.76
C–15	208.23	207.90	207.29	208.12	69.92	117.31	119.35	25.70	23.71	22.89	23.69	25.65
C–16	42.45*	42.36	42.17	42.38	38.12	35.87	35.10	27.03	28.78	27.92	28.71	27.01
C–17	50.79	50.76	50.73	50.80	54.69	57.03	58.44	56.72	54.64	55.81	54.44	56.50
C–18	18.97	19.00	19.03	18.97	19.56	15.65	16.45	18.44	11.25	11.80	11.26	18.45
C–19	14.52	14.52	14.51	14.52	14.34	20.49	14.21	14.39	19.86	14.77	19.86	14.39
C–20	34.50	34.37	34.07	34.28	34.19	33.85	33.93	34.22	36.02	35.94	35.76	33.95
C–21	19.21	19.16	18.88	19.13	18.96	18.81	18.88	19.01	18.66	18.78	18.38	18.74
C–22	35.81	31.55	27.50	35.67	35.70	31.78	31.76	31.62	31.75	31.73	27.86	27.71
C–23	23.49	29.12	40.69	24.44	24.53	29.34	29.35	29.28	29.44	29.39	40.95	40.82
C–24	39.35	63.27	202.33	124.53	124.90	63.57	63.58	63.59	63.56	63.58	203.24	203.18
C–25	27.96			131.42	131.13							
C–26	22.53			25.70	25.71							
C–27	22.74			17.65	17.63							
C–28	28.37	28.37	28.35	28.36	28.36	27.98	28.03	28.33	27.92	28.27	27.92	28.33
C–29	16.73	16.73	16.71	16.73	16.79	16.56	16.49	16.77	16.49	16.72	16.49	16.77
Acetate	21.30	21.31	21.28	21.30	21.32	21.32	21.34	21.34	21.33	21.34	21.33	21.33
	170.99	171.02	170.98	170.98	171.01	170.98	171.01	171.06	171.02	171.04	171.00	171.04

[a] ^{13}C NMR chemical shifts (±0.03 ppm) measured at 125 MHz in $CDCl_3$ solution (20–80 mm) at 22°C. Assignments marked by an asterisk may be interchanged within a column.

(100%; M^+), 426 (3%; M–H_2O), 411 (7%; M–H_2O–CH_3), 384* (12%; M–CH_3COOH), 369 (22%; M–CH_3COOH–CH_3), 341* (12%; $C_{23}H_{33}O_2$), 339 (16%), 297 (8%; M–CH_3COOH-SC), 279 (13%; M–CH_3COOH–SC–H_2O), 250 (22%; ion B), 235 (22%, ion B–CH_3), 217 (8%), and 135* (91%; $C_{10}H_{15}$); high resolution MS, calcd. for $C_{28}H_{44}O_4$ 444.3239, found 444.3247; IR, ν_{max} 3512, 2941, 2870, 1730, 1693, 1620, 1246, 1084, and 1034 cm^{-1}; UV, λ_{max} 259 nm (ε 14,400); ^1H NMR, Table 1; ^{13}C NMR, Table 2.

*3β-Acetoxy-15-oxo-4,4-dimethyl-5α-chol-8(14)-en-24-al (**VII**).* To oxalyl chloride (172 μl; 1.97 mmol) in CH_2Cl_2 (6 ml) at −50°C under argon was slowly added dimethyl sulfoxide (310 ml; 4.37 mmol) in CH_2Cl_2 (1 ml). After stirring for 2 min, **VI** (320 mg; 0.72 mmol) in CH_2Cl_2 (2 ml) was added dropwise. After stirring of the mixture for an additional 1 h at −50°C, triethylamine (1.2 ml) was added. The mixture was maintained at −50°C for 5 min and, after warming to room temperature, a saturated NH_4Cl solution (15 ml) was added. The resulting mixture was extracted with methyl *tert*-butyl ether (MTBE; 200 ml) and the organic extract was washed twice with a saturated NH_4Cl solution (15 ml portions), 3 times with a saturated cupric sulfate solution (15 ml portions), twice with a saturated NaCl solution (15 ml portions) and then dried over anhydrous sodium sulfate. The residue obtained upon evaporation of the solvent under reduced pressure was subjected to MPLC on a silica gel column (30 cm × 2.5 cm). Using 5% acetone in hexane as the eluting solvent, fractions (22 ml) were collected every 4 min. The contents of fractions 27 to 60 were combined to give, after evaporation of the solvent under reduced pressure, the desired aldehyde **VII** as a white solid (286 mg; 90% yield), MP 199–200°C; single component (R_f 0.63) on TLC (solvent, 40% ethyl acetate in hexane); MS, 442 (85%; M^+), 424* (37%; M–H_2O), 409 (2%; M–H_2O–CH_3), 382* (13%; M–CH_3COOH), 367* (17%, M–CH_3COOH–CH_3), 339* (20%; M–SC–H_2O), 297* (7%; M–SC–CH_3COOH), 279* (13%; M–SC–CH_3COOH-H_2O), 248* (18%, ion B), 233* (16%; ion B–CH_3), 230* (13%; $C_{16}H_{22}O$), and 135* (100%; $C_{10}H_{15}$); high resolution MS, calcd. for $C_{28}H_{42}O_4$ 442.3083, found 442.3086; IR, ν_{max} 2944, 2710, 1728, 1697, 1626, 1364, 1246, and 1032 cm^{-1}; UV λ_{max} 259 nm (ε 14,400); ^1H NMR, Table 1; ^{13}C NMR, Table 2.

*3β-Acetoxy-4,4-dimethyl-5α-cholesta-8(14),24-dien-15-one (**VIII**).* To a cold slurry of isopropyltriphenylphosphonium iodide (503 mg; 1.16 mmol) in tetrahydrofuran (3 ml) was added n-butyllithium (300 μl; 0.70 mmol) at 0°C under argon. The resulting red solution was stirred for 15 min and then

a portion (70%) was added dropwise to a solution of **VII** (190 mg; 0.43 mmol) in tetrahydrofuran (THF; 2 ml) at –78°C. After stirring for 2 h at 0°C, the mixture was poured into water and extracted 3 times with ether (100 ml portions). The combined ether extracts were washed twice with brine (20 ml portions), dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting residue was subjected to MPLC on a silica gel column (50 cm × 1 cm) using 2% acetone in hexane as the eluting solvent (fraction volume, 22 ml). The contents of fractions 4 to 19 were combined to give, after evaporation of the solvent under reduced pressure, **VIII** as a white solid (150 mg; 75% yield); MP 128.0–129.5°C; single component (R_f 0.90) on TLC (solvent, 25% ethyl acetate in hexane) and single component (t_R 8.20 min) on reversed phase HPLC; MS, 468 (100%; M^+), 453* (4%; M–CH_3), 408* (15%; M–CH_3COOH), 393* (25%; M–CH_3COOH–CH_3), 365* (9%, $C_{26}H_{37}O$), 339 (6%), 323 (2%), 297* (15%; M–CH_3COOH–SC–2H), 274* (14%; ion B), 259* (8%; ion B–CH_3), 243 (10%), 227 (5%), 165* (64%; $C_{11}H_{17}O$), 135* (80%; $C_{10}H_{15}$), 109 (35%), and 69 (43%); high resolution MS calcd. for $C_{31}H_{48}O_3$ 468.3603, found 468.3607; IR, ν_{max}2947, 1732, 1703, 1694, 1622, and 1244 cm^{-1}; UV, λ_{max} 259 nm (ε 14,700); ^{1}H NMR, Table 1; ^{13}C NMR, Table 2.

*3β-Acetoxy-4,4-dimethyl-5α-cholesta-8(14),24-dien-15β-ol (**IX**) and 3β-acetoxy-4,4-dimethyl-5α-cholesta-8(14),24-dien-15α-ol (**X**).* To **VIII** (75 mg; 0.16 mmol) in ethanol (20 ml) was added sodium borohydride (120 mg; 3.2 mmol). After stirring at room temperature for 2 h, the mixture was poured into water (20 ml) and extracted 3 times with MTBE (100 ml portions). The combined organic extracts were washed twice with brine (25 ml portions), dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting residue was subjected to MPLC on a silica gel column (50 cm × 1 cm) using 2% acetone in hexane as the eluting solvent; 22 ml fractions were collected. The contents of fractions 96 to 105 were combined and, after evaporation of the solvent under reduced pressure, gave the 15β-hydroxy-$\Delta^{8(14),24}$-diene **IX** (61 mg; 81% yield); MP 138.5-140.0°C; single component (R_f 0.72) on TLC (solvent, 25% ethyl acetate in hexane) and a single component (t_R 7.22 min) on reversed phase HPLC; MS, 470 (0.2%; M^+), 452* (100%; M–H_2O), 437* (28%; M–H_2O–CH_3), 409 (3%), 392* (9%; M–CH_3COOH–H_2O), 381* (6%; $C_{28}H_{41}$), 367 (26%), 340* (19%; $C_{23}H_{32}O_2$), 325 (6%), 288 (14%), 257* (11%; $C_{19}H_{29}$), 227* (6%; $C_{17}H_{23}$), 145* (15%; $C_{11}H_{13}$); high resolution MS calcd. for $C_{31}H_{50}O_3$ 470.3760, found 470.3751; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2.

The contents of fractions 108–115 were combined to give, after evaporation of the solvent under reduced pressure, the 15α-hydroxy-$\Delta^{8(14),24}$-diene **X** (4 mg; 5% yield) which showed a single component (t_R 6.83 min) on reversed phase HPLC; ^{1}H NMR, Table 1.

*3β-Acetoxy-4,4-dimethyl-5α-cholesta-8,14,24-triene (**XI**).* The 15β-hydroxy-$\Delta^{8(14),24}$-diene **IX** (55 mg; 0.12 mmol) in $CHCl_3$ (10 ml) was treated with sulfuric acid (20 μl) at room temperature with stirring for 5 min. Sodium bicarbonate (~30 mg) was added, and the mixture was diluted with CH_2Cl_2 (100 ml) and washed 3 times with brine (10 ml portions), dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting residue was subjected to MPLC on a silica gel column (25 cm × 1 cm) using 1% acetone in hexane as the eluting solvent; fractions (7 ml) were collected every 2 min. The contents of fractions 5–7 were combined to give, after evaporation of the solvent under reduced pressure, a white solid (50 mg) which showed a single component (R_f 0.79) on TLC (solvent, 10% ethyl acetate in hexane) and a single component (t_R 24.69 min) on reversed phase HPLC. However, analytical Ag^+-HPLC showed one major component (95%) accompanied by several minor, less polar components (1–2% each). Accordingly, the crude product was subjected to semipreparative Ag^+-HPLC (250 mm × 10 mm column) using 5% acetone in hexane as the eluting solvent (3 ml per min) with multiple injections of ~10 mg per injection and UV monitoring (210 nm) to give the $\Delta^{8,14,24}$ steryl acetate **XI** (45 mg; 85% yield); MP 137–138°C (lit. 126–128.5°C (3), 139–140°C (24)); MS, 452* (100%; M^+), 437* (25%; M–CH_3), 392* (4%; M–CH_3COOH), 377* (33%; M–CH_3COOH–CH_3), 367* (23%; $C_{25}H_{35}O_2$), 340 (14%), 325 (7%), 288 (17%), 279* (8%; M–CH_3COOH–SC–2H), 265* (7%; $C_{20}H_{25}$), and 159* (11%; $C_{12}H_{25}$); high resolution MS, calcd. for $C_{31}H_{48}O_2$ 452.3654, found 452.3653; IR, ν_{max} 2961, 2936, 2676, 1736, 1640, 1464, 1451, 1366, 1246, 1032, 1001, 990, 974, 941, 826, and 801 cm^{-1}; ^{1}H NMR, **Table 3**; ^{13}C NMR, **Table 4**. As noted above, the purity of **XI** was ~95% by Ag^+-HPLC. ^{1}H NMR analysis indicated a purity of ~90%, with small amounts of its $\Delta^{8,14,25}$ (3.5%), $\Delta^{6,8(14),24}$ (1%), and $\Delta^{7,14,24}$ (1–2%) isomers and two or three unidentified components (1–3% each). Repetition of the Ag^+-HPLC (250 mm × 10 mm column; elution with 5% acetone in hexane; ~10 mg portions) showed multiple components with the following t_R values (min): 10.8 (1.3%), 11.8 (0.7%), 26.4 (0.2%), 37.6 (0.2%), 49.0 (0.5%), and 58.6 (96%). The major component, corresponding to **XI**, showed a melting point (137–138°C) identical to that obtained prior to Ag^+-HPLC.

A similar reaction was carried out with sterol **IX** (190 mg; 0.40 mmol) in chloroform (15 ml) and sulfuric acid (5 μl), with stirring for 15 min at room temperature. Workup and MPLC, as described above, gave **XI** (~180 mg; ~98% yield) as a white solid which was characterized by ^{1}H NMR. The purity of **XI** was ~95%, accompanied by its $\Delta^{7,14,24}$ isomer (4%) and ~1–2% of unidentified material. In this case, none of the $\Delta^{6,8(14),24}$ or $\Delta^{8,14,25}$ isomers were detected.

*4,4-Dimethyl-5α-cholesta-8,14,24-trien-3β-ol (**I**).* The $\Delta^{8,14,24}$-steryl acetate **XI** (40 mg; 0.088 mmol) was heated with 10% KOH in 95% ethanol (3 ml) for 2 h at 70°C. Water (3 ml) was added and the resulting mixture was extracted 4 times with MTBE (20 ml portions). The combined organic extracts were washed twice with brine (10 ml portions), dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue was subjected to MPLC on a silica gel column (50 cm × 1 cm) using 2% acetone in hexane as the eluting solvent. Fractions (~20 ml) were col-

TABLE 3. ^{1}H NMR chemical shifts for unsaturated 4,4-dimethylsterols and acetate derivatives[a]

	$\Delta^{8,14,24}$ 3β-ol **I**	$\Delta^{8,24}$ 3β-ol II	$\Delta^{8,14}$ 3β-ol III	$\Delta^{7,14}$ 3β-ol **IV**	$\Delta^{8,14,24}$ 3β-acetate **XI**	$\Delta^{8,24}$ 3β-acetate **XXI**	$\Delta^{8(14),24}$ 3β-ol **XXII**
H–1α	1.324	1.279	1.324	1.169	1.387	1.340	1.161
H–1β	1.848	1.749	1.848	1.851	1.845	1.745	1.667
H–2α	1.723†	1.688	1.721†	1.638†	1.762†	1.717	1.655†
H–2β	1.632†	1.596	1.631†	1.600†	1.654†	1.622	1.552†
H–3α	3.243	3.239	3.244	3.245	4.488	4.495	3.260
H–5α	1.216	1.125	1.217	1.188	1.302	1.209	0.985
H–6α	1.765	1.678	1.765	2.05	1.750	1.662	1.617
H–6β	1.555	1.504	1.555†	2.05	1.563	1.508	1.304
H–7α	2.307†	1.929†	2.308†	5.825	2.314†	1.936†	1.710
H–7β	2.190†	2.058†	2.192†		2.188†	2.050†	2.449
H–9α				1.736			1.613†
H–11α	2.17	2.06*	2.18	1.528†	2.17	2.05*	1.519†
H–11β	2.17	2.04*	2.16	1.462†	2.17	2.03*	1.444†
H–12α	1.379	1.363	1.378†	1.284	1.389	1.374	1.097
H–12β	2.016	1.960	2.017	2.024	2.013	1.955	1.929
H–14α		2.014†				2.028†	
H–15α	5.360	1.578	5.360	5.508	5.363	1.578	2.185
H–15β		1.271†				1.267†	2.245
H–16α	2.360	1.893	2.348	2.307	2.362†	1.895	1.827
H–16β	2.064	1.317†	2.055	1.916	2.063†	1.315†	1.373
H–17α	1.517	1.139	1.502	1.573†	1.526	1.147	1.122
H–18	0.810	0.592	0.808	0.818	0.807	0.590	0.838
H–19	1.034	0.986	1.033	0.847	1.054	1.006	0.752
H–20	1.630	1.398†	1.604†	1.578†	1.629	1.397†	1.470†
H–21	0.955	0.940	0.932	0.921	0.955	0.940	0.947
H–22R	1.444	1.407†	1.372†	1.36	1.443	1.406†	1.452†
H–22S	1.093	1.030	1.044†	1.036†	1.097	1.032	1.106
H–23R	1.880	1.849	1.363†	1.36	1.879	1.849	1.861
H–23S	2.046	2.022	1.175†	1.177†	2.047	2.023	2.017
H–24R	5.108	5.092	1.115†	1.112†	5.108	5.092	5.096
H–24S			1.155†	1.154†			
H–25			1.526	1.526			
H–26	1.687	1.682	0.867	0.867	1.686	1.682	1.683
H–27	1.607	1.602	0.870	0.871	1.607	1.601	1.601
H–28	1.017	0.998	1.017	0.988	0.898	0.876	1.005
H–29	0.832	0.812	0.832	0.894	0.903	0.883	0.813
Ac					2.055	2.049	

[a] See footnotes for Table 1.

lected. The contents of fractions 29–46 were combined to give, after evaporation of the solvent under reduced pressure, **I** (38 mg; 105% yield) as a white solid; ~96% purity by Ag^+-HPLC and ~91–93% purity by ^{1}H NMR (containing the $\Delta^{6,8(14),24}$ (1%), $\Delta^{8,14,25}$ (3%), and $\Delta^{7,14,24}$ (2%) isomers along with unidentified material (1–2%). Further purification by Ag^+-HPLC (250 mm × 10 mm column; elution with 10% acetone in hexane) gave **I** (t_R 47.0 min) accompanied by three minor components (t_R values of 14.2, 36.6, and 140 min). Recrystallization of **I** gave an analytical sample; MP 135-137°C (lit. 119–121°C (24)); single component (R_f 0.80) on TLC (solvent, 40% ethyl acetate in hexane), a single component (t_R 15.5 min) on reversed phase HPLC, and a purity of 99% on Ag^+-HPLC (t_R 18.6 min) using 9.1% acetone in hexane as the eluting solvent and a purity of 98–99% by ^{1}H NMR; MS, 410* (100%; M^+), 395* (33%; M–CH_3), 377* (19%; M–CH_3–H_2O), 328* (11%; $C_{23}H_{36}O$), 325* (26%; $C_{23}H_{33}O$), 313* (11%; $C_{22}H_{33}O$), 298* (10%; $C_{21}H_{30}O$), 283 (12%), 265 (4%), and 246 (18%); high resolution MS, calcd. for $C_{29}H_{46}O$ 410.3549, found 410.3555; UV, λ_{max} 249 nm (ϵ 18,600); ^{1}H NMR, Table 3; ^{13}C NMR, Table 4.

Partial ^{1}H NMR data for the minor components 4,4-dimethyl-5α-cholesta-6,8(14),24-trien-3β-ol and 4,4-dimethyl-5α-cholesta-8,14,25-trien-3β-ol, obtained by the Ag^+-HPLC described above, were as follows.

4,4-Dimethyl-5α-cholesta-6,8(14),24-trien-3β-ol (~80% purity): δ 0.678 (s, H–19), 0.826 (s, H–29), 0.888 (d, 0.6 Hz, H–18), 0.958 (d, 6.6 Hz, H–21), 1.063 (s, H–28), 1.602 (br s, H–27), 1.684 (br q, ~1.2 Hz, H–26), 1.896 (br t, ~2.8 Hz, H–5α), 1.997 (dt, 12.5, 3.4 Hz, H–12β), 3.301 (dd, 11.1, 2.5 Hz, H–3α), 5.095 (br t, ~6.8 Hz, H–24), 5.616 (dd, 10.1, 2.3 Hz, H–6), 6.232 (dd, 10.1, 3.2 Hz, H–7).

4,4-Dimethyl-5α-cholesta-8,14,25-trien-3β-ol (~80% purity): δ 0.810 (d, 0.4 Hz, H–18), 0.832 (s, H–29), 0.945 (d, 6.6 Hz, H–21), 1.017 (s, H–28), 1.033 (d, 0.7 Hz, H–19), 1.217 (dd, 12.5, 2.1 Hz, H–5α), 1.715 (br dd, 1.4, 0.9 Hz, H–27), 1.848 (dt, 13.0, 3.6 Hz, H–1β), 2.348 (ddd, 16.0, 7.3, 3.2 Hz, H–16α), 3.244 (dd, 11.8, 4.6 Hz, H–3α), 4.667 (m, $W_{1/2}$ ~5 Hz, H–26), 4.689 (m, $W_{1/2}$ ~5 Hz, H–26), 5.357 (br dd, ~3, ~2 Hz, H–15).

*3β-Acetoxy-4,4-dimethyl-5α-chola-8,14-dien-24-ol (**XII**), 3β-acetoxy-4,4-dimethyl-5α-chola-7,14-dien-24-ol (**XIII**), 3β-acetoxy-4,4-dimethyl-5α-chola-6,8(14)-dien-24-ol (**XIV**), and 3β-acetoxy-4,4-dimethyl-5α-chola-8(14),15-dien-24-ol (**XV**).* To the 3β-acetate derivative of the 4,4-dimethyl-$\Delta^{8(14)}$-15-keto-C_{24}-3β,24-diol **VI** (170 mg; 0.38

TABLE 4. ^{13}C NMR chemical shifts for unsaturated 4,4-dimethylsterols and acetate derivatives[a]

	$\Delta^{8,14,24}$ 3β-ol **I**	$\Delta^{8,24}$ 3β-ol **II**	$\Delta^{8,24}$ 3β-ol **II**[b]	$\Delta^{8,14}$ 3β-ol **III**	$\Delta^{7,14}$ 3β-ol **IV**	$\Delta^{8,14,24}$ 3β-acetate **XI**	$\Delta^{8,24}$ 3β-acetate **XXI**	$\Delta^{8(14),24}$ 3β-ol **XXII**
C–1	36.04	35.73	35.8	36.05	37.71	35.67	35.38	37.03
C–2	27.87	27.87	28.0	27.88	27.45	24.19	24.19	27.69
C–3	78.70	78.95	79.0	78.70	79.18	80.66	80.92	79.22
C–4	39.02	38.91	38.9	39.02	38.60	37.90	37.81	38.93
C–5	50.39	50.19	50.2	50.39	49.38	50.43	50.26	54.23
C–6	18.27	18.42	18.4	18.28	23.60	18.15	18.30	21.88
C–7	27.87	28.44	28.5	27.88	120.72	27.66	28.27	30.14
C–8	122.77	127.93	128.0	122.77	133.58	122.73	127.91	126.12
C–9	141.75	135.80	135.8	141.74	52.35	141.48	135.56	51.42
C–10	37.75	36.94	37.0	37.75	35.01	37.60	36.82	37.78
C–11	21.08	22.03	22.1	21.09	20.46	21.10	22.02	19.46
C–12	37.03	36.94	29.7	37.04	39.95	36.94	36.87	37.31
C–13	44.97	42.08	42.1	44.95	46.34	44.94	42.05	42.64
C–14	150.99	51.86	51.9	151.02	151.75	150.93	51.78	142.09
C–15	117.36	23.78	23.8	117.38	119.35	117.45	23.75	25.73
C–16	35.89	28.78	28.8	35.92	35.14	35.89	28.77	26.97
C–17	57.13	54.74	54.8	57.23	58.65	57.07	54.70	56.83
C–18	15.67	11.24	11.3	15.66	16.44	15.67	11.25	18.43
C–19	20.45	19.83	19.8	20.45	14.20	20.49	19.86	14.37
C–20	33.88	36.07	36.0	34.05	34.12	33.86	36.06	34.23
C–21	18.78	18.62	18.6	18.85	18.91	18.76	18.61	18.99
C–22	35.99	36.01	36.1	36.09	36.06	35.98	36.01	35.85
C–23	24.64	24.78	24.8	23.76	23.77	24.61	24.77	24.61
C–24	125.12	125.19	125.2	39.50	39.49	125.11	125.19	125.17
C–25	131.00	130.93	130.9	28.00	28.00	131.00	130.91	130.96
C–26	25.73	25.72	25.7	22.55	22.55	25.73	25.72	25.72
C–27	17.63	17.63	17.6	22.80	22.81	17.63	17.62	17.63
C–28	28.00	27.94	27.9	28.00	28.06	27.99	27.93	28.40
C–29	15.42	15.35	15.4	15.42	15.32	16.57	16.49	15.64
Ac						21.34	21.34	
Ac						170.99	170.99	

[a] ^{13}C NMR chemical shifts (±0.03 ppm) measured at 125 MHz in $CDCl_3$ solution (20–80 mm) at 22°C.
[b] Data from reference 1 (±0.1 ppm).

mmol) in ethanol (40 ml) was added sodium borohydride (500 mg; 13 mmol). After stirring for 2 h at room temperature, the mixture was poured into water (50 ml) and extracted 3 times with ethyl acetate (100 ml portions). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to give 3β-acetoxy-4,4-dimethyl-5α-chol-8(14)-ene-15ξ,24-diol, which showed one major (~99%) component (R_f 0.67) and a minor (~1%) component (R_f 0.57) on TLC (solvent, 25% acetone in hexane). The 15,24-diol was dissolved in $CHCl_3$ (50 ml) and treated with sulfuric acid (40 μl) for 15 min at room temperature. $NaHCO_3$ (400 mg) was added and, after stirring for 2 min, the mixture was filtered through a short silica gel column (2 cm × 1 cm) using $CHCl_3$ (50 ml) and acetone (20 ml) as the eluting solvents. The collected eluate was evaporated to dryness under reduced pressure, and the resulting residue was subjected to MPLC on a silica gel column (50 cm × 1 cm). Using 5% ethyl acetate in hexane as the eluting solvent, fractions (20 ml) were collected every 5 min. The contents of fractions 62 to 103 were combined to give, after evaporation of the solvent under reduced pressure, a white solid (163 mg). The crude product showed one component (R_f 0.4) on TLC (solvent, 25% acetone in hexane) and it showed a single component (t_R 87.2 min) on capillary GC analysis of its TMS ether derivative on a 60 m DB-5 column. However, analysis by Ag^+-HPLC (300 mm × 3.2 mm), with 15% acetone in hexane as the eluting solvent, showed 4 components with the following retention times: 22.0 min (4%), 25.6 min (3%), 41.1 min (82%), and 45.9 min (10%). ^1H NMR analysis of the product also showed the presence of 4 components as indicated by methyl and olefinic proton chemical shifts corresponding to the following isomers: $\Delta^{8,14}$ **XII** (87%), $\Delta^{7,14}$ **XIII** (9%), $\Delta^{8(14),15}$ **XV** (3.5%), and $\Delta^{6,8(14)}$ **XIV** (0.5%).

To resolve the individual isomers, the mixture was, in ~4-5 mg portions, subjected to semipreparative Ag^+-HPLC (250 mm × 10 mm) using 15% acetone in hexane as the eluting solvent and to apply the sterol mixture to the column) at a flow rate of 3 ml per min. Elution of the individual components was monitored by UV (210 nm) and by analysis of aliquots of fractions (3 ml) on an analytical Ag^+-HPLC column (300 mm × 3.2 mm) using 15% acetone in hexane as the eluting solvent. After a total of 40 injections, the following pure products were obtained (in order of elution): $\Delta^{6,8(14)}$-steryl acetate **XIV** (1 mg; 0.6% yield), $\Delta^{8(14),15}$-steryl acetate **XV** (5 mg; 3% yield), $\Delta^{8,14}$-steryl acetate **XII** (141 mg; 86% yield), and $\Delta^{7,14}$-steryl acetate **XIII** (14 mg; 8.5% yield). Each of the compounds showed a single component (99%) on analytical Ag^+-HPLC (300 mm × 3.2 mm; solvent, 15% acetone in hexane). Retention times for **XIV**, **XV**, **XII**, and **XIII** were

22.0, 25.2, 41.1, and 45.1 min, respectively. Compound **XII** melted at 172.5-173°C. The MS of each of the compounds showed a molecular ion at *m/z* 428. High resolution MS for **XIV**, **XV**, **XII**, and **XIII** showed exact masses of 428.3288, 428.3294, 428.3291, and 428.3294, respectively; calcd. for $C_{28}H_{44}O_3$ 428.3290. The MS of **XIV**, **XV**, **XII**, and **XIII**, shown in **Table 5**, were similar in the high mass range, but with notable different relative abundances for a number of the ions. ^{1}H NMR spectral data for each of the four isomers are presented in Table 1, and ^{13}C NMR spectral data for **XII** and **XIII** are presented in Table 2.

*3β-Acetoxy-4,4-dimethyl-5α-chol-8(14)-en-24-ol (**XVI**), 3β-acetoxy-4,4-dimethyl-5α-chol-8-en-24-ol (**XVII**), and 3β-acetoxy-4,4-dimethyl-5α-chol-7-en-24-ol (**XVIII**).* The $\Delta^{8,14}$ diene **XII** (95 mg; 0.22 mmol) in ethyl acetate (20 ml; freshly distilled from K_2CO_3) was hydrogenated for 24 h at one atmosphere at room temperature over a freshly prepared (25) Raney nickel catalyst (5 g). The mixture was applied to a silica gel column (10 cm × 1 cm) which was washed with additional ethyl acetate (300 ml). The total eluant was evaporated to dryness under reduced pressure to give a white solid (92 mg) which showed a single component (R_f 0.4) on TLC (solvent, 25% ethyl acetate in hexane). GC–MS analysis of the TMS derivative of the crude product showed three components, each with molecular ions at *m/z* 502, with retention times of 87.6 min (50%), 92.3 min (43%), and 98.7 min (7%). Ag^+-HPLC (300 mm × 3.2 mm; solvent, 9.1% 2-propanol in hexane at 1 ml per min) showed 3 components with retention times of 4.31 min, 6.15 min, and 7.16 min. ^{1}H NMR analysis of the crude product showed the following chemical shifts for the upfield methyl region: 0.841 ($\Delta^{8(14)}$; 53%), 0.595 (Δ^8; 39%), and 0.523 (Δ^7; 8%). The crude product was subjected to semipreparative Ag^+-HPLC (250 mm × 10 mm) using 5% acetone in hexane as the eluting solvent at 3 ml per min. Fractions (3 ml) were collected each min. The elution of the components was monitored by UV detection (210 nm) and by analytical Ag^+-HPLC (300 mm × 3.2 mm) of aliquots of the fractions. After 25 injections, the 3 components were obtained in a pure state: the $\Delta^{8(14)}$-24-ol **XVI** (45 mg; 47% yield), MP 182.0–182.5°C; the Δ^8-24-ol **XVII** (35 mg; 37% yield), MP 220.0–220.5°C; and the Δ^7-24-ol **XVIII** (9 mg; 9% yield), MP 202.0–203.0°C. Compounds **XVI**, **XVII**, and **XVIII** each showed single components on Ag^+-HPLC (300 mm × 3.2 mm; solvent 9.1% acetone in hexane) with t_R values of 36.5 min, 40.9 min, and 48.9 min, respectively, and purities of 99% by ^{1}H NMR. MS data for **XVI**, **XVII**, and **XVIII** are presented in **Table 6**, and high resolution MS showed 430.3460, 430.3450, and 430.3443, respectively (calcd. for $C_{28}H_{46}O_3$ 430.3447). ^{1}H NMR and ^{13}C NMR data for **XVI**, **XVII**, and **XVIII** are presented in Tables 1 and 2, respectively.

*3β-Acetoxy-4,4-dimethyl-5α-chol-8-en-24-al (**XIX**) and 3β-acetoxy-4,4-dimethyl-5α-chol-8(14)-en-24-al (**XX**).* To a cooled (–50°C) solution of oxalyl chloride (0.5 ml; 1 mmol; 2 m in CH_2Cl_2) in CH_2Cl_2 (2 ml) was added dimethyl sulfoxide (0.15 ml; 2.1 mmol) in CH_2Cl_2 (0.2 ml) over 3 min under argon. After stirring for 2 min, the Δ^8 24-ol **XVII** (29 mg; 0.068 mmol) in CH_2Cl_2 (1 ml) was added over 5 min. After stirring for 1 h, triethylamine (0.6 ml; 4.3 mmol) was slowly added. After stirring for 5 min, the mixture was warmed to room temperature, and a saturated solution of NH_4Cl (3 ml) and MTBE (150 ml) was successively added. The separated MTBE layer was washed twice with a saturated NH_4Cl solution (3 ml por-

TABLE 5. Mass spectral data for 3β-acetoxy-4,4-dimethyl-5α-chola-8,14-dien-24-ol (**XII**), 3β-acetoxy-4,4-dimethyl-5α-chola-7, 14-dien-24-ol (**XIII**), 3β-acetoxy-4,4-dimethyl-5α-chola-6, 8(14)-dien-24-ol (**XIV**), and 3β-acetoxy-4,4-dimethyl-5α-chola-8(14), 15-dien-24-ol (**XV**)

m/z		**XII** $\Delta^{8,14}$	**XIII** $\Delta^{7,14}$	**XIV** $\Delta^{6,8(14)}$	**XV** $\Delta^{8(14),15}$
428	M^+	100*	100*	91*	100*
426	M–2H	22*	18*	12*	22*
413	M–CH_3	21*	27*	48*	13*
368	M–CH_3COOH	22*	12*	54*	11*
353	M–CH_3COOH–CH_3	78*	28*	86*	19*
351		11	5	13	5
341	M–SC	18*	58*	92*	76*
327	$C_{22}H_{31}O_2$	10	20*	19	34*
325	$C_{23}H_{33}O$	15	6	11	5*
299	$C_{21}H_{31}O$	8*	5	25*	5*
297	$C_{21}H_{29}O$	14	2	12	3*
281	M–SC–CH_3COOH	28*	31*	100*	41*
267	$C_{20}H_{27}$	14	7	20	8*
266	M–SC–CH_3COOH–CH_3	18*	4	11	3
265		10	4	11	5
259	$C_{18}H_{21}O$	11*	2	4	3*
255	$C_{19}H_{27}$	6*	7*	56*	5*
253	$C_{19}H_{25}$	8*	4	12*	5*
239	$C_{18}H_{23}$	13*	7*	33*	7*
233	$C_{16}H_{25}O$	5	6*	6	40*
227	$C_{17}H_{23}$	14*	14*	93*	6*
225	$C_{17}H_{21}$	12*	6*	21*	8*
215	$C_{16}H_{23}$	6*	4	7	9*
213	$C_{16}H_{21}$	14*	9*	25*	14*
211	$C_{16}H_{19}$	14*	9*	32*	8*

Ions designated with an asterisk showed exact mass values on high resolution MS which were within 3 millimass units of the calculated values for the proposed ions.

TABLE 6. Mass spectral data for 3β-acetoxy-4,4-dimethyl-5α-chol-8(14)-en-24-ol (**XVI**), 3β-acetoxy-4,4-dimethyl-5α-chol-8-en-24-ol (**XVII**), and 3β-acetoxy-4,4-dimethyl-5α-chol-7-en-24-ol (**XVIII**)

m/z		**XVI** $\Delta^{8(14)}$	**XVII** Δ^8	**XVIII** Δ^7
430	M^+	100*	100*	100*
415	M–CH_3	32*	36*	22*
370	M–CH_3COOH	24*	24*	10*
355	M–CH_3COOH–CH_3	31*	41*	16*
343	M–SC	24*	24*	12*
327	$C_{23}H_{35}O$	12*	11	4*
301		11	13	7
288		11	10	3
283	M–SC–CH_3COOH	27*	29*	46*
257	$C_{19}H_{29}$	23*	23*	8*
241	$C_{18}H_{25}$	34*	39*	18*
235	$C_{16}H_{27}O$	23*	25*	9*
229	$C_{17}H_{25}$	10*	13*	14*
221	$C_{15}H_{25}O$	18*	18*	8*
219	$C_{15}H_{23}O$	15*	14*	5*
213	$C_{16}H_{21}$	11*	14*	6*

Ions designated with an asterisk showed exact mass values on high resolution MS which were within 3 millimass units of the calculated values for the proposed ions.

tions), three times with a saturated cupric sulfate solution (5 ml portions), and once with brine (5 ml), and then dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting residue was applied to a silica gel column (10 cm × 1 cm) using hexane (10 ml) and then a mixture (3:10:87) of acetone, CH_2Cl_2, and hexane (100 ml) as the eluting solvents, and fractions (5 ml) were collected. The contents of fractions 7 to 10 were combined and evaporated to dryness under nitrogen to give the Δ^8-C_{24}-24-aldehyde **XIX** (26 mg; 90% yield); MP 213.5–214.5°C; single component (R_f 0.7) on TLC (solvent, 25% acetone in hexane), single component (t_R 31.2 min) on Ag^+-HPLC (300 mm × 3.2 mm; solvent, 3% acetone in hexane), and a purity of 99% by ^{1}H NMR; MS, 428* (100%; M^+), 413* (32%; M–CH_3), 400* (23%; M–CO), 368* (28%; M–CH_3COOH), 353* (44%; M–CH_3COOH–CH_3), 343* (24%; M–SC), 325 (22%), 301* (12%; $C_{20}H_{29}O_2$), 283 (29%; M–SC–CH_3COOH), 257 (22%), 241 (43%), 135* (71%; $C_{10}H_{15}$); high resolution MS, calcd. for $C_{28}H_{44}O_3$ 428.3290, found 428.3286; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2.

Oxidation of the $\Delta^{8(14)}$-C_{24}-24-ol **XVI** was carried out in a similar fashion. Oxalyl chloride (1.05 ml; 2.1 mmol; 2 M in CH_2Cl_2) in CH_2Cl_2 (3 ml) was cooled to −50°C under argon. Dimethyl sulfoxide (0.31 ml; 4.4 mmol) was added over 3 min. **XVI** (38 mg; 0.088 mmol) in CH_2Cl_2 (1 ml) was added over 5 min. After stirring for 1 h, triethylamine (1.14 ml; 8.2 mmol) was slowly added. The mixture was worked up as described above and then subjected to silica gel column (10 cm × 1 cm) chromatography using hexane (10 ml) and 5% acetone in hexane (100 ml) as the eluting solvent (fraction size, 5 ml). The contents of fractions 7 to 9 were combined to give, after evaporation of the solvent, the $\Delta^{8(14)}$-C_{24}-24-aldehyde **XX** (35 mg; 92% yield); MP 162.5-163.5°C; single component (R_f 0.7) on TLC (solvent, 25% acetone in hexane), single component (t_R 22.74 min) on Ag^+-HPLC (300 mm × 3.2 mm; solvent, 3% acetone in hexane), and a purity of 99% by ^{1}H NMR; MS, 428* (100%; M^+), 413* (28%; M–CH_3), 400* (20%; M–CO), 368* (23%; M–CH_3COOH), 353* (32%; M–CH_3COOH–CH_3), 343* (23%; M–SC), 325 (18%), 301 (9%), 283* (25%; M–SC-CH_3COOH), 257 (19%), 241 (34%), 135* (65%; $C_{10}H_{15}$); high resolution MS, calcd. for $C_{28}H_{44}O_3$ 428.3290, found 428.3289; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2.

*3β-Acetoxy-4,4-dimethyl-5α-cholesta-8,24-diene (**XXI**).* To isopropyltriphenylphosphonium iodide (840 mg; 1.94 mmol) in THF (5 ml) was added n-butyllithium (0.6 mmol) in hexane (380 μl) at 0°C under argon. After stirring for 15 min, an aliquot (600 μl) of the resulting mixture was added to **XIX** (21 mg; 0.049 mmol) in THF (1 ml) at −78°C. The mixture was warmed to 0°C and, after stirring for 2 h, poured into water (30 ml) and extracted 4 times with MTBE (50 ml portions). The combined organic extracts were washed once with brine (20 ml), dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue was applied to a silica gel column (8 cm × 0.8 cm) and the column was eluted with a 1:1:50 mixture of acetone, CH_2Cl_2, and hexane (fraction size, 4 ml). The contents of fractions 2 to 6 were combined and evaporated to dryness under nitrogen to give **XXI** (23 mg; 103% yield); MP 137–139°C (lit. 133–134°C (26), 128–132°C (27)); single component (R_f 0.9) on TLC (solvent 25% acetone in hexane), single component (t_R 28.56 min) on Ag^+-HPLC (300 mm × 3.2 mm; solvent, 3% acetone in hexane), and a purity of 99% by ^{1}H NMR; MS, 454* (100%; M^+), 439 (25%; M–CH_3), 394* (7%; M–CH_3COOH), 379* (15%; M–CH_3COOH–CH_3), 341 (5%; M–SC–2H), 259 (10%), 257 (9%), 245 (6%), 241 (15%), and 135 (30%); high resolution MS, calcd. for $C_{31}H_{50}O_2$ 454.3811, found 454.3809; ^{1}H NMR, Table 3; ^{13}C NMR, Table 4.

*4,4-Dimethyl-5α-cholesta-8,24-dien-3β-ol (**II**).* The $\Delta^{8,24}$ steryl acetate **XXI** (11 mg; 0.024 mmol) was heated with 15% KOH in 95% ethanol (3 ml) for 2 h at 60°C. Water (3 ml) was added, and the resulting mixture was extracted 4 times with MTBE (12 ml portions). The combined organic extracts were washed with brine (3 ml), dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue was subjected to MPLC on a silica gel column (8 cm × 0.8 cm) using 4% acetone in hexane as the eluting solvent (fraction size, 3 ml). The contents of fractions 3 to 5 were combined and evaporated to dryness under nitrogen to give **II** (10 mg; 100% yield) as a white solid; MP 158–159°C (lit. 139.5–140°C (26), 136–138.5°C (27), 128–129°C (24)); single component (R_f 0.4) on TLC (solvent, 25% acetone in hexane), and a purity of 99% by ^{1}H NMR; MS, **Fig. 4**; high resolution MS, calcd. for $C_{29}H_{48}O$ 412.3705, found 412.3702; ^{1}H NMR, Table 3; ^{13}C NMR, Table 4.

*4,4-Dimethyl-5α-cholesta-8(14),24-dien-3β-ol (**XXII**).* The $\Delta^{8(14)}$-C_{24}-24-aldehyde **XX** (29 mg; 0.068 mmol) was treated with the Wittig reagent and the crude product was isolated and subjected to silica gel column chromatography as described above for the preparation of the $\Delta^{8,24}$ diene **XXI** (except for the use of an older sample of the isopropyltriphenylphosphonium iodide employed in the preparation of the Wittig reagent). The crude product, a mixture of **XXII** and its acetate and acetoacetate derivatives (as indicated by ^{1}H and ^{13}C NMR), was heated with 15% KOH in 95% ethanol (3 ml) for 2 h at 60°C. Water (3 ml) was added and the resulting mixture was extracted 4 times with MTBE (12 ml portions). The combined organic extracts were washed with brine (3 ml), dried over anhydrous sodium sulfate, and evaporated to dryness under nitrogen to give **XXII** (10 mg; 36% yield) as a white solid; MP 140–141°C; single component (R_f 0.4) on TLC (solvent, 25% acetone in hexane), and a purity of 99% by ^{1}H NMR; MS, Fig. 4; high resolution MS, calcd. for $C_{29}H_{48}O$ 412.3705, found 412.3701; ^{1}H NMR, Table 3; ^{13}C NMR, Table 4.

Chromatography of 3β-acetate derivatives of 4,4-dimethylsterols

Presented in **Table 7** are capillary GC and Ag^+-HPLC data for the acetate derivatives of unsaturated 4,4-dimethylsterols with double bonds in the Δ^5, $\Delta^{5,7}$, $\Delta^{8,24}$, $\Delta^{6,8(14)}$, $\Delta^{7,14}$, $\Delta^{8,14}$, and $\Delta^{8,14,24}$ positions. Also presented are previously reported (28) TLC data for some of the same compounds on silica gel-$AgNO_3$ plates. Whereas a number of the compounds showed clearly different retention times on GC, essentially no separation of the $\Delta^{8,14}$ and $\Delta^{5,7}$ iso-

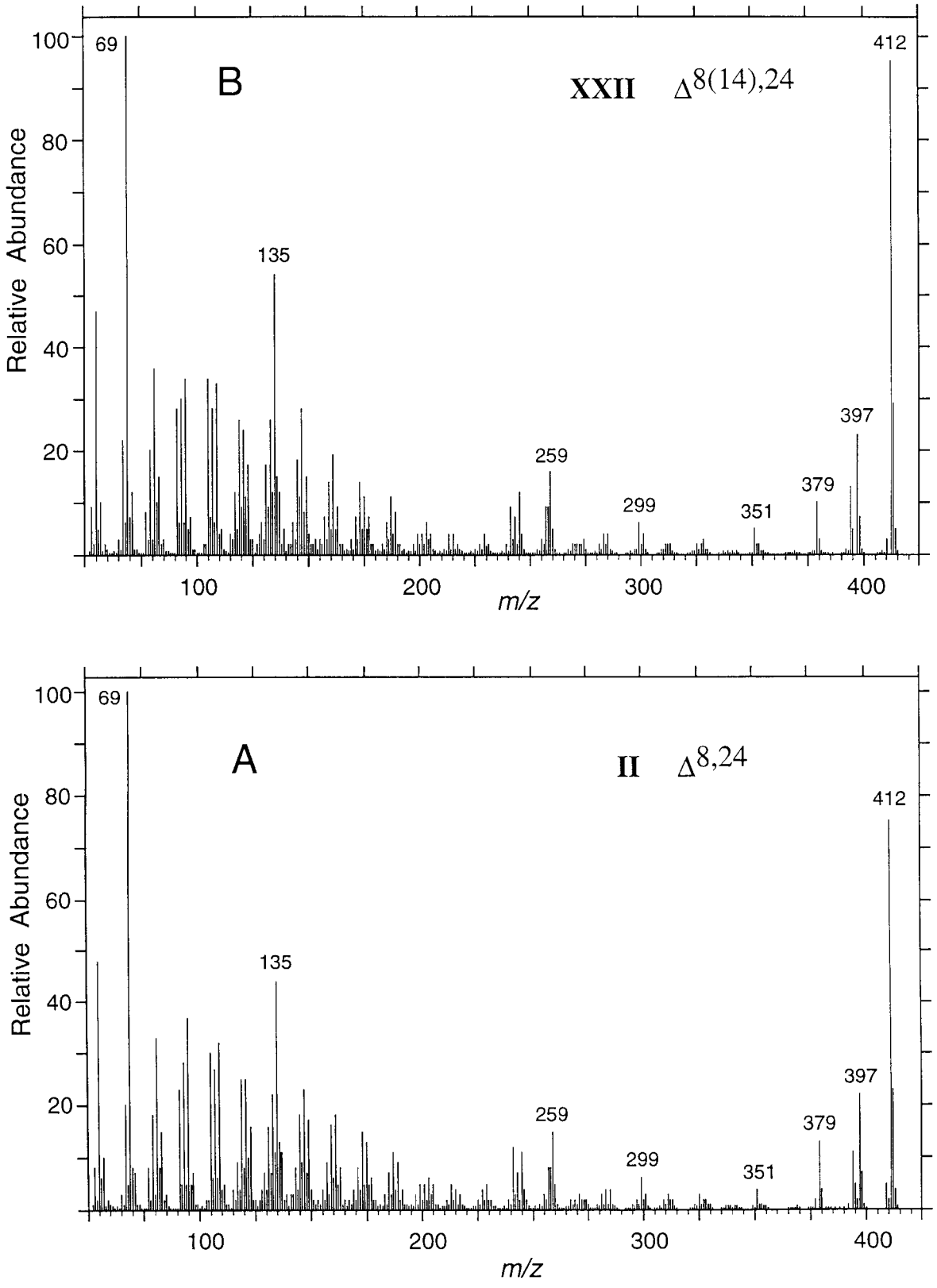

Fig. 4. Mass spectra of (A) 4,4-dimethyl-5α-cholesta-8,24-diene (**II**) and (B) its $\Delta^{8(14),24}$ isomer (**XXII**).

mers was observed and only partial resolution of the same compounds from the $\Delta^{7,14}$ isomer was observed. Ag^{+}-HPLC was found to be considerably more powerful for the separation of the concerned compounds with the exception of the case of the $\Delta^{5,7}$ and $\Delta^{8,24}$ isomers. The value of the Ag^{+}-HPLC over simple TLC on silica gel-$AgNO_3$ plates is illustrated by the lack of any resolution of the $\Delta^{8,14}$ and $\Delta^{7,14}$ isomers in the latter system.

TABLE 7. Chromatography of 3β-acetate derivatives of unsaturated 4,4-dimethylsterols

Double Bond Position	Ag^+-HPLC[a] (RRT)	GC[b] (RRT)	TLC (silica gel–$AgNO_3$)
			R_f
Δ^5	1.00	3.33	0.58
$\Delta^{5,7}$	1.87	3.49	0.53
$\Delta^{8,24}$	1.90	3.95	—
$\Delta^{6,8(14)}$	4.06	3.67	0.46
$\Delta^{7,14}$	8.67	3.56	0.41
$\Delta^{8,14}$	9.21	3.48	0.41
$\Delta^{8,14,24}$	>23[c]	3.86	—

[a] Three hundred mm × 4.6 mm; solvent, 3% acetone in hexane, RRT, retention time relative to 3β-acetoxy-4,4-dimethylcholest-5-ene (absolute retention time, 7.48 min).

[b] Thirty m DB5 column, injector and column temperature, 250°C; nitrogen carrier gas with head pressure of 1.1 kg/cm^2; RRT, retention time relative to 5α-cholestane (absolute retention time, 13.62 min).

[c] Not eluted within 200 min.

As noted previously, Ag^+-HPLC also proved extremely useful in the purification of the 3β-acetate derivatives of unsaturated 3β,24-dihydroxy C_{24} compounds. Retention times (min) for the $\Delta^{6,8(14)}$, $\Delta^{8(14),15}$, $\Delta^{8,14}$, and $\Delta^{7,14}$ diunsaturated steroids on Ag^+-HPLC (300 mm × 3.2 mm; solvent, 15% acetone in hexane) were 22.0, 25.2, 41.1, and 45.9, respectively. Using 9.1% acetone in hexane as the eluting solvent, retention times (min) for the $\Delta^{8(14)}$, Δ^8, and Δ^7 monounsaturated isomers were 36.5, 40.9, and 48.9, respectively.

Effects of synthetic sterols on meiosis

In the first experiment (**Fig. 5**, panel A), 49% of the oocytes underwent germinal vesicle breakdown (GVB) in medium containing 3.5 mm hypoxanthine. In contrast, 85% and 73% of the oocytes cultured under the same conditions, but treated at a concentration of 3 μg/ml, with the 4,4-dimethyl-$\Delta^{8,14}$ sterol (**III**) or the 4,4-dimethyl-$\Delta^{7,14}$ sterol (**IV**), respectively, underwent GVB. However, two other 4,4-dimethylsterols, i.e., 4,4-dimethylcholesta-5,7-dien-3β-ol and 4,4-dimethylcholest-5-en-3β-ol, did not promote the resumption of meiosis. Moreover, the 20R,22R- and 20S,22S- isomers of 20,22-dihydroxycholesterol and the 22R- and 22S-isomers of 22-hydroxycholesterol had no significant effect on meiosis under the same conditions. In the second experiment (Fig. 5, panel B), both 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol (**II**) and 4,4-dimethyl-5α-cholesta-8(14),24-dien-3β-ol (**XXII**) were found to promote GVB. In the third experiment (**Fig. 6**), the 4,4-dimethyl-$\Delta^{8,14}$-sterol (**III**) and the 4,4-dimethyl-$\Delta^{7,14}$-sterol (**IV**), at 3 μg/ml, had a significant effect on meiosis, as did the 4,4-dimethyl-$\Delta^{8,14,24}$-sterol (**I**). At this concentration, the extent of GVB was significantly higher with **I** than with **III** or **IV**.

DISCUSSION

In 1995, Byskov et al. (1) reported that four 4,4-dimethylsterols, i.e., 4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol (**I**), 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol (**II**), 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol (**III**), and 4,4-dimethyl-5α-cholest-8-en-3β-ol, affected meiosis in cultured cumulus-enclosed and naked mouse oocytes. Compounds **I** and **II** were isolated from human follicular fluid and bull testicular tissue, respectively (see below). The other two sterols, containing $\Delta^{8,14}$ and Δ^8 unsaturated moieties, were prepared by chemical synthesis and were reported to show purities of 98.5% and 97.8%, respectively, on the basis of reversed phase HPLC and MS data. These results alone would not, in our experience, preclude the presence of closely related double bond isomers. However, it was stated that ^1H and ^{13}C NMR spectral data were those expected. For evaluation of their actions on the cultured oocytes, the sterols were reported to have been sonicated (3 times for 1 min) in culture medium prior to addition to the cells. This procedure, which could easily result in the formation of oxidation products of the various unsaturated sterols, was used because "the material could not be dissolved in alcohol," the latter observation being, in our experience, unexpected for the concerned sterols (and confirmed herein). In determination of the meiosis activa-

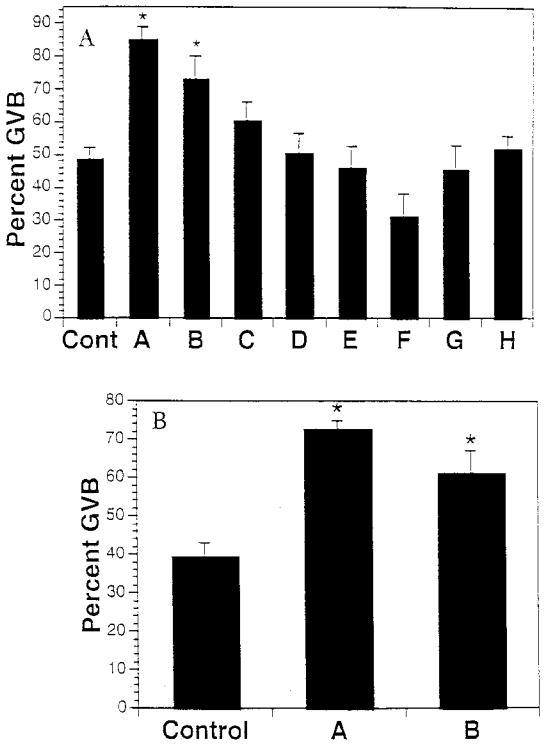

Fig. 5. The ability of various sterols (3 μg/ml) to promote germinal vesicle breakdown (GVB) in the presence of hypoxanthine (3.5 mm). The bars indicate the mean percentage (±SEM) of oocytes that underwent GVB in 4 independent experiments. (*) indicates significant difference ($P \leq 0.05$) from the control. Panel A. Cont, control; A, 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol (**III**); B, 4,4-dimethyl-5α-cholesta-7,14-dien-3β-ol (**IV**); C, 4,4-dimethylcholesta-5,7-dien-3β-ol; D, 4,4-dimethylcholest-5-en-3β-ol; E, (20R,22R) cholest-5-ene-3β,20,22-triol; F, (20S,22S)cholest-5-ene-3β,20,22-triol; G, (22R)cholest-5-ene-3β,22-diol; H, (22S)cholest-5-ene-3β,22-diol. Panel B. A, 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol (**II**); B, 4,4-dimethyl-5α-cholesta-8(14),24-dien-3β-ol (**XXII**).

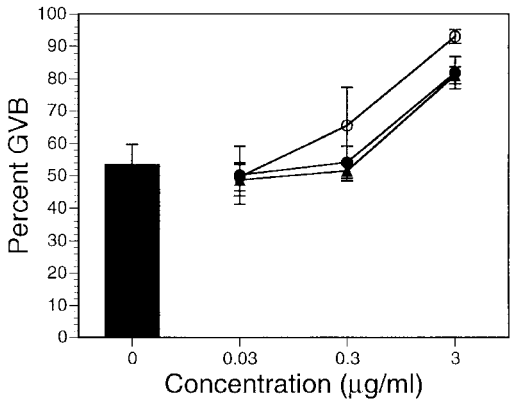

Fig. 6. The ability of 4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol (**I**; open circles), 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol (**III**; solid circles), and 4,4-dimethyl-5α-cholesta-7,14-dien-3β-ol (**IV**; solid triangles) to promote GVB in the presence of hypoxanthine (3.5 mm). The solid bar indicates the mean value for the control. The results (mean ± SEM for 4 independent experiments) showed that **I**, **III**, and **IV** (at 3 μg/ml) were significantly different from the control and that **I** was significantly greater than **III** and **IV**.

tion by the various sterols, considerable experimental variation was encountered. Lanosterol, cholesterol, and ergosterol were reported to result in no activation of meiosis under the conditions studied. In 1998, Byskov et al. (29) reported that two other sterols, i.e., 4β-methyl-5α-cholesta-8,24-dien-3β-ol and 5α-cholesta-8,24-dien-3β-ol, also affected meiosis in oocytes.

The 4,4-dimethylsterols **I**, **II**, and **III**, along with 4,4-dimethyl-5α-cholest-8-en-3β-ol are potential intermediates in sterol biosynthesis (8). **I** and **III** are products of the 14α-demethylation of lanosterol and 24,25-dihydrolanosterol, respectively (2, 4–7). Moreover, and of potential relevance to the matter of the reported effects of these sterols on meiosis, the conversion of lanosterol to **I** by rat ovary has recently been reported to be stimulated by gonadotropin of pregnant mare's serum (9). It should also be noted that **I** has been reported to activate gene expression for the nuclear orphan receptor LXR_{α} (10).

For exploration of these interesting and potentially important matters, we have pursued the chemical syntheses of 4,4-dimethylsterols **I** and **II**. We (21) and others (30, 31) have previously described the preparation of the 4,4-dimethyl-$\Delta^{8,14}$-sterol **III**. The syntheses of **I** and **II** represent more formidable challenges, requiring the introduction of the 4,4-dimethyl functionality, the provision of the $\Delta^{8,14}$ and Δ^{8} double bonds of **I** and **II**, respectively, and the introduction of the Δ^{24} double bond. A number of synthetic routes to **I** and **II** can be envisioned with different orders of introduction of the various functionalities. However, considerations of economy and practicality dictate evaluation of the availability and costs of potential starting materials. Cholesterol, available at relatively low cost from commercial sources, can be readily obtained in a high state of purity. Ergosterol and various plant sterols are more costly and more difficult to obtain in a high state of purity.

Our synthesis of **I** (Fig. 2) used the 4,4-dimethyl-$\Delta^{8(14)}$-15-ketosteryl ester **V**, obtained from cholesterol, as the starting material (21). A critical step in the synthesis is based on our development of conditions permitting a remarkably efficient and specific oxidation of the saturated side chain of 3β-acetoxy-5α-cholest-8(14)-en-15-one with a mixture of trifluoroacetic anhydride, hydrogen peroxide, and sulfuric acid to give 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (14, 32), a C_{24} synthon already protected at C-3 from which the desired C_8 side chain with various substituents in the side chain can be constructed (15, 19, 33). Oxidation of the 4,4-dimethyl-$\Delta^{8(14)}$-15-ketosteryl ester **V** proceeded in gratifyingly high (73%) yield to give the C_{24} 24-hydroxy-$\Delta^{8(14)}$-15-ketosteryl acetate (**VI**) from which the corresponding aldehyde **VII** was prepared in high (90%) yield. Wittig olefination of **VII** with isopropyltriphenylphosphonium iodide and butyllithium gave the $\Delta^{8(14),24}$-15-ketosteryl ester **VIII** in 75% yield. Reduction of **VIII** with sodium borohydride gave a mixture which was resolved by silica gel MPLC to give the 15β-hydroxy-$\Delta^{8(14)}$-steryl ester **IX** (81% yield) and the corresponding 15α-hydroxy isomer (5% yield). Acid treatment of **IX** gave, after semipreparative Ag^{+}-HPLC, the $\Delta^{8,14,24}$-steryl acetate **XI** in 85% yield. Saponification of **XI** gave, after purification by silica gel MPLC, **I** in essentially quantitative yield.

The 4,4-dimethyl-$\Delta^{8,24}$ sterol **II** was prepared as outlined in Fig. 3. The C_{24} 24-hydroxy-4,4-dimethyl-$\Delta^{8(14)}$-15-ketosteryl acetate **VI** was reduced with sodium borohydride to give the corresponding 15ξ,24-dihydroxy compound which, upon treatment with acid, gave a mixture of four dienes **XII**-**XV** as judged by Ag^{+}-HPLC and ^{1}H NMR. Purification by Ag^{+}-HPLC resolved the desired $\Delta^{8,14}$ acetate **XII** (86% yield) and three other components, i.e., the $\Delta^{7,14}$ isomer **XIII** (8.5% yield), the $\Delta^{6,8(14)}$ isomer **XIV** (0.6% yield), and the $\Delta^{8(14),15}$ isomer **XV** (3% yield). Catalytic hydrogenation of the $\Delta^{8,14}$ acetate **XII** gave a mixture of 3 isomeric components as judged by GC–MS, Ag^{+}-HPLC, and ^{1}H NMR. Ag^{+}-HPLC permitted the isolation of the 3 components: Δ^{8} isomer **XVII** (37% yield), $\Delta^{8(14)}$ isomer **XVI** (47% yield), and the Δ^{7} isomer **XVIII** (9% yield). The C_{24} Δ^{8}-24-hydroxy compound **XVII** was oxidized to the corresponding aldehyde **XIX** (90% yield) which, upon Wittig olefination, gave the $\Delta^{8,24}$ steryl acetate **XXI** in essentially quantitative yield. Saponification of **XXI** gave the desired $\Delta^{8,24}$ sterol **II** (100% yield). The overall yield for the conversion of the C_{27} 4,4-dimethyl-$\Delta^{8(14)}$-15-ketosteryl acetate **V** to the 4,4-dimethyl-$\Delta^{8,24}$-sterol **II** was 21%.

Oxidation of the $\Delta^{8(14)}$-24-hydroxy compound **XVI** to the corresponding aldehyde **XX** followed by Wittig olefination gave, after saponification, the previously undescribed 4,4-dimethyl-$\Delta^{8(14),24}$ sterol **XXII**.

The starting material **V** and the intermediates and major byproducts in the syntheses of $\Delta^{8,14,24}$-, $\Delta^{8,24}$-, and $\Delta^{8(14),24}$-4,4-dimethylsterols (**I**, **II**, and **XXII**) were characterized by melting point, MS, high resolution MS, ^{1}H and ^{13}C NMR, and, when appropriate, UV spectral analysis. Minor byproducts were, in general, characterized only by NMR supplemented, in some cases, by MS.

The MS of compounds **V**, **VI**, **VII**, and **VIII** showed the expected ions in the high mass region along with ions characteristic of $\Delta^{8(14)}$-15-ketosteroids (13–19). The MS of the 3β-acetates of the isomeric 4,4-dimethyl diunsaturated C_{24} 24-alcohols containing double bonds in the $\Delta^{8,14}$ (**XII**), $\Delta^{7,14}$ (**XIII**), $\Delta^{6,8(14)}$ (**XIV**), and $\Delta^{8(14),25}$ (**XV**) positions were very similar, differing only in the relative abundances of the individual ions with the $\Delta^{6,8(14)}$ compound **XIV** being least similar in relative ion abundances to the other isomers (Table 5). The MS of the 3β-acetates of the isomeric 4,4-dimethyl monounsaturated C_{24} 24-alcohols containing double bonds in the $\Delta^{8(14)}$ (**XVI**), Δ^{8} (**XVII**), and Δ^{7} (**XVIII**) positions were very similar, differing only in the relative abundances of the individual ions and with virtually identical MS for the $\Delta^{8(14)}$ and Δ^{8} isomers (**XVI** and **XVII**, respectively). It is also noteworthy that the MS of the 3β-acetates of $\Delta^{8(14)}$ and Δ^{8} isomers of the 4,4-dimethyl monounsaturated C_{24} aldehydes (**XIX** and **XX**, respectively) were essentially identical as were the MS of the $\Delta^{8,24}$ and $\Delta^{8(14),24}$ isomers of the 4,4-dimethyl C_{27} sterols, **II** and **XXII**, respectively (Figure 4). These findings clearly demonstrate that MS alone provides no basis for distinguishing between the 4,4-dimethylsterols with double bonds in the $\Delta^{8,24}$ and $\Delta^{8(14),24}$ positions.

Tables 1–4 present high precision ^{13}C and ^{1}H NMR chemical shift data and assignments for each of the carbon atoms and protons of the starting material (**V**) and of each of the intermediates and major byproducts in the chemical syntheses of **I**, **II**, and **XXII**. These chemical shift data and individual assignments not only provide important structural information for the concerned compounds but also provide a basis for comparisons with previously reported data and with data to be obtained for isolated or synthetic sterols in this series.

In 1989, Dolle et al. (24) described the synthesis of **I** and **II** from the benzoate ester of 3β-hydroxy-4,4-dimethylergosta-8(14),22-dien-15-one which itself can be prepared from ergosterol (34). The $\Delta^{8(14),22}$-15-ketosteryl ester was converted to **I** through an eleven-step synthesis, which involved production of the corresponding C-22 aldehyde by ozonolysis, elaboration of the desired C_8 side chain with oxygen functions at C-22 and C-24, removal of the oxygen functionality at C-22, generation of the $\Delta^{8,14}$ diene system, and finally, introduction of the Δ^{24} double bond from the trifluoroacetate ester of the 4,4-dimethyl-$\Delta^{8,14}$-24-hydroxysterol. The reported overall yield of **I** from the 4,4-dimethyl-$\Delta^{8}(^{14),22}$-15-ketosteryl ester was high (~53%). However, many intermediates were not isolated and characterized. Moreover, the melting point of **I** reported (119–121°C) was notably lower than that reported herein (135–137°C). Limited spectral data for **I** were presented. ^{1}H NMR data, obtained at lower precision than those described herein (Table 3), were presented for the following protons: 3α, 15, 18, 19, 21, 24, 26, 27, 28, and 29. The chemical shifts and assignments were in general agreement with those presented in Table 3 with the exception of an interchange of the assignments for H-18 and H-19. The synthesis of **II** described by Dolle et al. (24) was also a multistep synthesis in which many intermediates were not isolated and characterized. The overall yield of **II** from the $\Delta^{8(14),22}$-15-ketosteryl ester was ~18%. The reported melting point for **II** (128–129°C) was considerably less than that reported herein (158–159°C). Very limited spectral data were presented for **II**. ^{1}H NMR data were reported only for the 3α (δ 3.30) and 24 (δ 5.21) protons which were not in good agreement with the data for **II** presented herein (Table 3). Maitra, Mohan, and Sprinson (35) previously described the preparation of labeled **II** by incubation of labeled mevalonate with 10,000 × supernatant fraction of rat liver homogenate preparations in the presence of added arsenite and lanosterol. The isolated biosynthetic **II** was characterized as its acetate derivative by its MS with major ions at *m/z* 454, 439, 394, 379, and 341 in the high region of the spectrum (as described herein) and by ^{1}H NMR with reported peaks and assignments at δ 0.59 (18–CH_3), 0.88 (19–CH_3 and 4α–CH_3), 0.94 (21–CH_3), 1.01 (4β–CH_3), 1.60, 1.68 (26– and 27–CH_3), and 2.05 (CH_3CO_2). Our results (Table 3) indicate that the assignments of Maitra et al. (35) for H–19 and H–29 (4β–CH_3) should be reversed.

The 4,4-dimethyl-$\Delta^{8,14,24}$ sterol **I** (designated by Byskov et al. (1) as FF-MAS) and the 4,4-dimethyl-$\Delta^{8,24}$ sterol **II** (designated as T-MAS) were reported to have been obtained by n-heptane extraction of human follicular fluid and bull testicular tissue, respectively, followed by three HPLC purification steps (two reversed phase and one normal phase). Evidence for the structure of the 4,4-dimethyl-$\Delta^{8,14,24}$ sterol **I** was limited to presentation of its mass spectrum, but with no comparison with that of an authentic sample. Insufficient material was available for NMR analysis. Evidence for the structure of the 4,4-dimethyl-$\Delta^{8,24}$ sterol **II** was based upon the combination of its mass spectrum and the results of ^{1}H and ^{13}C NMR, again without comparisons with data for an authentic sample. The NMR data for **II** were reported to indicate the presence of two impurities (~10% lanosterol and 1% of material of unknown structure). Only ^{13}C NMR data for **II** were presented. The assignment of structure to **II** was based upon comparison of its ^{13}C NMR spectral data with our previously presented data for lanosterol (36) and zymosterol (5α-cholesta-8,24-dien-3β-ol) (37). The availability of synthetic **II**, as described herein, permits comparison of the ^{13}C NMR data reported for the isolated sample of **II** (Table 4) with data obtained at higher precision for synthetic **II**, prepared in a high state of purity as described herein. The data for the two samples are in reasonable agreement with the exception of the signal at δ 29.7 which was assigned by Byskov et al. (1) to C-12. The signal at δ 29.7 corresponds to that observed in compounds with a long-chain alkane moiety.

As noted previously, Byskov et al. (29) reported that 4β-methyl-5α-cholesta-8,24-dien-3β-ol activated meiosis in oocytes. This sterol was reportedly isolated after incubation of a yeast, *Kluyveromyces bulgaricus* A3410, in the presence of amphotericin B (29). The sterol in question was isolated by HPLC. Evidence for its identity was based upon MS, with its MS data reported as "identical with those of 4β-methylzymosterol as recorded in the National Bureau

of Standards library." To our knowledge, the only previous descriptions of the 4β-methyl-$\Delta^{8,24}$ sterol have been those concerning a 4-methyl-$\Delta^{8,24}$ sterol isolated from the skin of rats treated with triparanol (38–40). It should be noted that a subsequent study (41) demonstrated that the physical data presented for the sterol isolated from skin corresponds to the 4α-methyl isomer, i.e., 4α-methyl-5α-cholesta-8, 24-dien-3β-ol.

The availability of the synthetic 4,4-dimethylsterols with double bonds in the $\Delta^{8,14,24}$, $\Delta^{8,24}$, $\Delta^{8(14),24}$, $\Delta^{8,14}$, $\Delta^{7,14}$, $\Delta^{5,7}$, and Δ^{5} positions permitted evaluation of their effects on meiosis in mouse oocytes. The synthetic $\Delta^{8,14,24}$ and $\Delta^{8,24}$ sterols (**I** and **II**, respectively) caused a resumption of meiosis as noted for the isolated sterols by Byskov et al. (1), as did the 4,4-dimethyl-$\Delta^{8,14}$ sterol **III**. We also report, for the first time, that the corresponding 4,4-dimethyl-$\Delta^{7,14}$ and $\Delta^{8(14),24}$ sterols (**IV** and **XXII**, respectively) also resulted in a resumption of meiosis. The $\Delta^{8,14,24}$ sterol **I** was more potent than the $\Delta^{8,14}$ and $\Delta^{7,14}$ sterols (**III** and **IV**, respectively). Not all 4,4-dimethylsterols showed significant activity; the $\Delta^{5,7}$ and Δ^{5} sterols were inactive under the conditions studied.

The 4,4-dimethyl-$\Delta^{8,14,24}$-sterol **I** has been reported to be a positive regulator of the orphan nuclear receptor LXR_{α} (10). This orphan receptor is also activated by several oxygenated sterols, most notably by (22R)-22-hydroxycholesterol (10, 22), (20S)-20-hydroxycholesterol (10, 22), and (20R,22R)-20,22-dihydroxycholesterol (22). In one study (10), the unnatural 22S isomer of 22-hydroxycholesterol was reported to show high potency in activating LXR_{α}, whereas in another study (22), it had no effect. These combined findings, coupled with our demonstration that synthetic **I** had a significant effect on meiosis in the mouse oocytes, prompted evaluation of the effects of the 20R,22R- and 20S,22S-isomers of 20,22-dihydroxycholesterol and of the 22R- and 22S-isomers of 22-hydroxycholesterol on the resumption of meiosis. No effect on meiosis was observed with any of these compounds.

As noted previously, Ag^{+}-HPLC was found to be extraordinarily valuable in the purification of sterols differing only in the location of olefinic bonds and in the assessment of purity of the various synthetic sterols. This methodology should prove valuable in studies of the metabolism of the 4,4-dimethylsterols found to activate meiosis (i.e., **I**, **II**, **III**, **IV**, and **XXII**). Moreover, the new chemical syntheses of **I**, **II**, and **XXII** presented herein represent potentially valuable routes for the preparation of isotopically labeled analogs (^{3}H and ^{14}C) of high specific activity through the use of the appropriately labeled Wittig reagent. Such labeled analogs could prove valuable for studies of the metabolism of the sterols as well as for studies of their possible interaction with potential receptors such as the orphan receptor LXR_{α}.

In summary, novel chemical syntheses of **I** and **II** and the $\Delta^{8(14),24}$ analog **XXII** have been presented. These synthetic 4,4-dimethylsterols, along with their $\Delta^{8,14}$ and $\Delta^{7,14}$ analogs, also prepared by chemical synthesis, caused a resumption of meiosis in mouse oocytes in the presence of hypoxanthine (3.5 mM). Further studies are clearly indicated to extend these findings towards an understanding of the mechanism(s) and physiological significance of the in vitro effects of the sterols on meiosis by the concerned sterols. The availability of synthetic sterols of defined structure and purity, as well as information regarding their chromatographic, MS, and ^{1}H and ^{13}C NMR spectral properties, should facilitate future investigations of these matters.[3]

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[3]Since the submission of our manuscript, Grøndahl et al. (42) have also described that **I**, prepared by chemical synthesis, caused a resumption of meiosis in mouse oocytes incubated in the presence of hypoxanthine (3 mm). **I** was reported to have been synthesized "by a novel method": however, no information on this matter was provided. Grøndahl et al. (42) also reported that several oxysterols that affect the nuclear orphan receptor LXR_{α} had no effect on meiosis under the conditions studied.

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