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Sterol synthesis. Preparation and characterization of fluorinated and deuterated analogs of oxygenated derivatives of cholesterol

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Abstract

Oxygenated sterols, including both autoxidation products and sterol metabolites, have many important biological activities. Identification and quantitation of oxysterols by chromatographic and spectroscopic methods is greatly facilitated by the availability of authentic standards, and deuterated and fluorinated analogs are valuable as internal standards for quantitation. We describe the preparation, purification and characterization of 43 oxygenated sterols, including the 4β-hydroxy, 7α-hydroxy, 7β-hydroxy, 7-keto, and 19-hydroxy derivatives of cholesterol and their analogs with 25,26,26,26,27,27,27-heptafluoro (F_7) and 26,26,26,27,27,27-hexadeuterio (d_6) substitution. The 7α-hydroxy, 7β-hydroxy, and 7-keto derivatives of (25*R*)-cholest-5-ene-3β,26-diol (**1d**) and their 16,16-dideuterio analogs were also prepared. These d_2-26-hydroxysterols and [16,16-$^2H_2$]-(25*R*)-cholest-5-ene-3β,26-diol (**1e**) were synthesized from [16,16-$^2H_2$]-(25*R*)-cholest-5-ene-3β,26-diol diacetate (**2e**), which can be prepared from diosgenin. The highly specific deuterium incorporation at C-16 in **1e** and **2e** should be useful in mass spectral analysis of 26-hydroxycholesterol samples by isotope dilution methods. The Δ^5-3β,7α,26- and Δ^5-3β,7β,26-triols were regioselectively oxidized/isomerized to the corresponding Δ^4-3-ketosteroids with cholesterol oxidase. Also described are 5,6α-epoxy-5α-cholestan-3β-ol, its 5β,6β-isomer, cholestane-3β,5α,6β-triol, their F_7 and d_6 derivatives, and d_3-25-hydroxycholesterol, which was prepared from 3β-acetoxy-27-norcholest-5-en-25-one (**30**). The 43 oxysterols and most synthetic intermediates were isolated in high purity and characterized by chromatographic and spectroscopic methods,

Abbreviations: COSYDEC, f_1-decoupled $^1H-^1H$ correlation spectroscopy; COSY-DQF, $^1H-^1H$ correlation spectroscopy with double-quantum filtering; GC, gas chromatography; HMBC, heteronuclear multiple bond correlation; HPLC, high performance liquid chromatography; HSQC, heteronuclear single-quantum coherence; IR, infrared (spectroscopy); m.p., melting point; MPLC, medium pressure liquid chromatography; MS, mass spectrometry or mass spectrum; NMR, nuclear magnetic resonance (spectroscopy); NOE, nuclear Overhauser enhancement; SC, side chain; TBDMS, *tert*-butyldimethylsilyl; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, sodium salt; TLC, thin-layer chromatography; TMS, trimethylsilyl.

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including mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. Detailed mass spectral assignments are presented, and ^{1}H NMR stereochemical assignments are derived for the C-19 protons of 19-hydroxysterols and for the side-chain protons of **30**. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Oxygenated sterols; Deuterium-labeled 26-hydroxycholesterol; Alumina–silver nitrate chromatography; NMR; Mass spectrometry

1. Introduction

Oxysterols constitute a class of lipids of high interest in biology and medicine. For bioanalytical studies based upon GC-MS, we required labeled analogs of major oxygenated sterols. Described herein are syntheses of deuterium- and fluorine-substituted analogs of a number of oxysterols of high current biomedical interest. Also presented are detailed spectral (MS, ^{1}H and ^{13}C NMR, and, in most cases, IR) characterization of the synthetic sterols. Although deuterium-labeled analogs of some of the oxysterols have been prepared previously (Björkhem and Kallner, 1976; Javitt et al., 1981, 1982; Björkhem, 1986; Breuer and Björkhem, 1990; Shoda et al., 1993a; Breuer, 1995; Dzeletovic et al., 1995; Krut et al., 1997), little or no characterization of the sterols (or mixtures of same) were presented. Among new synthetic oxysterol analogs described herein are the 25,26,26,26,27,27,27-heptafluoro analogs of a number of oxysterols, (25*R*)-[16,16-$^2H_2$]cholest-5-ene-3β,26-diol, and the corresponding d_2 analogs of (25*R*)-3β,26-dihydroxycholest-5-en-7-one, (25*R*)-cholest-5-ene-3β,7α,26-triol, and (25*R*)-cholest-5-ene-3β,7β,26-triol.

2. Experimental procedures and results

2.1. Materials and methods

Melting points (m.p.) were measured with a Thomas–Hoover apparatus in sealed, evacuated capillary tubes. IR spectra were obtained from KBr pellets on a Mattson Galaxy 6020 Fourier-transform infrared spectrometer. TLC was carried out on aluminum-backed, silica gel 60 plates (EM Science, Gibbstown, NJ). Components of the plates were visualized after spraying with 5% ammonium molybdate in 10% sulfuric acid followed by heating. TLC solvent systems were: SS-1, ethyl acetate–hexane 1:9; SS-2, ethyl acetate–hexane 15:85; SS-3, ethyl acetate–hexane 2:8; SS-4, ethyl acetate–hexane 3:7; SS-5, ethyl acetate–hexane 1:1; SS-6, ethyl acetate–hexane 6:4; SS-7, methanol–chloroform 1:9. MPLC was done on glass columns; unless specified otherwise, columns were dry-packed with silica gel (230–400 mesh; EM Science), and fraction volumes were 20 ml. Low-resolution and high-resolution mass spectra were recorded after direct-inlet sample introduction on a VG ZAB-HF reverse-geometry double-sector instrument at 70 eV with an electron-impact ion source (200°C). Mass spectral data are given as m/z (relative abundance, suggested assignment or molecular formula). Relative abundances (for $m/z \geq 50$) are from low-resolution spectra, and exact masses are from high-resolution data. Exact masses are reported as the average of $\sim$five scans; standard deviations were typically 1–1.5 mmu (millimass units) for ions of $\geq 10\%$ relative abundance. Ions attributable to loss of CH_3, side chain (SC), CH_3COOH, H_2O, HBr, Br; $(CH_3)_3C(CH_3)_2SiOH$, C_4H_9 (of TBDMS ethers); CH_2O, CH_2OH, and CH_2O-2 (of 19-hydroxysterols), or combinations thereof are marked by an asterisk (*). Ions showing exact masses within ± 3.0 mmu of the indicated or implied assignments are marked by †. Bromine-containing ions are marked by §; only ions containing ^{79}Br are listed. NMR spectra were acquired as described previously (Swaminathan et al., 1993; Wilson et al., 1996) on a Bruker AMX500 (5–15 mM $CDCl_3$ solution at 25°C for ^{1}H; 10–120 mM $CDCl_3$ solution at 22°C for ^{13}C; $CHCl_3$ solution

at 25°C for ^2H, unless specified otherwise) or AC250 spectrometer ($CDCl_3$ solution at ~22°C for ^{19}F). NMR chemical shifts were referenced to $(CH_3)_4Si$ (^1H), $CDCl_3$ at δ_C 77.0 (^{13}C), $CDCl_3$ at δ_D 7.26 (^2H), and the downfield line of the 3:3:1:0.1 isotope pattern for $CFCl_3$ (^{19}F). Chemical shifts were corrected for strong coupling in 1D spectra by analogy with the analysis of AB spin systems and in COSYDEC spectra by adjustments based on spectral simulations with NMRSIM (Bruker Instruments, Billerica, MA). Signal assignments were made from HSQC, COSYDEC, COSY-DQF, and 1D spectra as described previously (Wilson et al., 1996). PCMODEL 7.0 (Serena Software; Bloomington, IN) was used for modeling of sterol structures by molecular mechanics and for predicting vicinal ^1H–^1H NMR coupling constants. The purity of sterol samples was judged by TLC and ^1H NMR (500 MHz spectrum; methyl region and δ_H 2.5–7.0 region). Stated purities are exclusive of traces of solvent and (in the case of **18a**, **18b**, **18c**, **19a**, **19b**, **19c**, and **21b**) non-steroidal long chain alkyl contaminants arising from NMR sample preparation techniques.

Butyl acetate, *N*-bromoacetamide, *m*-chloroperbenzoic acid (≥85% purity), hydrazine hydrate (85%), hydrazine hydrochloride, lead tetraacetate, lithium aluminum hydride, methyl iodide-d_3 (96% d_3, 4% d_2 by MS), potassium permanganate, pyridinium chlorochromate, L-Selectride (lithium tri-*sec*-butyl borohydride), selenium dioxide, and tetrabutylammonium fluoride were purchased from Aldrich Chemical Co. (Milwaukee, WI). TES buffer, cholesterol oxidase from *Streptomyces* sp., and catalase were purchased from Sigma Chemical Co. (St. Louis, MO). Alumina–silver nitrate for MPLC was prepared as described previously (Pascal et al., 1980) from neutral aluminum oxide (ICN Biomedicals; Costa Mesa, CA). $CDCl_3$ for NMR analysis was filtered through furnace-dried basic alumina prior to use. [26,26,26,27,27,27-$^2H_6$]cholest-5-en-3β-ol (**1c**; Medical Isotopes, Inc.; Pelham, NH; ≥99% purity by ^1H NMR; 93% d_6, 7% d_5 by MS), 3β-acetoxy-27-norcholest-5-en-25-one (**30**; Southeastern Biochemicals, Augusta, GA; ~95% purity by ^1H NMR), lithium aluminum deuteride (≥99% D, Isotech; Miamisburg, OH) were purchased and used without further purification. 25,26,26,26,27,27,27-Heptafluorocholest-5-en-3β-ol (**1b**; F_7-cholesterol) (Swaminathan et al., 1993), (25*R*)-bis(3β,26 -*tert* - butyldimethylsilyloxy)cholest - 5 - en -16-one (**9**) (Kim et al., 1989), (25*R*)-cholest-5-ene-3β,26-diol (**1d**) (Kim et al., 1989), and its diacetate **2d** (Siddiqui et al., 1992) were prepared as described previously and characterized as follows: **9**, m.p. 80–81°C, lit. 81.5–82.5°C (Kim et al., 1989); **1d**, m.p. 176–177°C, lit. 177–178°C (Kim et al., 1989), single component by TLC (R_f 0.65, SS-5); **2d**, m.p. 126.5–127°C, lit. 130.5–131.5°C (Siddiqui et al., 1992), single component by TLC (R_f 0.60, SS-2).

*2.2. 25,26,26,26,27,27,27-Heptafluorocholest-5-en-3β-ol acetate (**2b**), cholest-5-en-3β-ol acetate (**2a**), and [26,26,26,27,27,27-$^2H_6$]cholest-5-en-3β-ol acetate (**2c**)*

To a solution of F_7-cholesterol (**1b**; 100 mg, 0.20 mmol) in pyridine (1 ml) was added acetic anhydride (40 mg, 0.40 mmol). The reaction mixture was heated to reflux for 1 h, allowed to cool, and poured into ice-water (200 ml). The resulting precipitate was collected by filtration, washed with water, and dried in vacuo. Filtration through silica gel in a Pasteur pipette (elution with ethyl acetate–hexane 2:98) gave **2b** as a white solid (96 mg, 89% yield, 98% purity): m.p., 135–136°C (lit. 137–139°C (Carroll et al., 1998)); single component by TLC (R_f 0.50, SS-1); 98% purity by ^1H NMR (contains 1.5% (23*E*)-3β-acetoxy-25,26,26,26,27,27,27-heptafluorocholesta-5,23-diene); high-resolution MS, calcd. for $C_{27}H_{37}F_7$ (M − 60), 494.2783, found 494.2777; MS, 494*† (100), 479*† (16), 386† (7, $C_{19}H_{25}F_7$), 373† (19, $C_{18}H_{24}F_7$), 331† (5, $C_{15}H_{18}F_7$), 283† (4, M − CH_3COOH–$C_6H_6F_7$), 255*† (9), 239† (4, SC), 213† (10, $C_{16}H_{21}$), 147† (31, $C_{11}H_{15}$), 105† (27, C_8H_9); IR, 2947, 2905, 2874, 2852, 1736, 1467, 1440, 1377, 1346, 1313, 1278, 1246, 1222, 1134, 1037 cm^{-1}; ^1H NMR, Table 1; ^{13}C NMR, Table 2.

Similar acetylation of **1a** (2.5 g) gave **2a** (2.5 g, 90% yield): m.p. 114–115°C; single component by TLC (R_f 0.60, SS-1); MS, 368* (100, M − 60),

Table 1
^{1}H NMR chemical shifts of F_7-cholesterol acetate (**2b**), 26-hydroxy derivatives (**11**, **15**, **16d**, **18d**, **19d**), synthetic precursors (**26a**, **27a**, **28b**) to 19-hydroxysterols (**29b**), and 27-norketone **30**[a,b]

		26-Hydroxy derivatives									
	3β-OAc **2b**	16α-OH TBDMS **11**	Δ^{16} Acetate **15**	7-Keto Acetate **16d**	7α-OAc Acetate **18d**	7β-OAc Acetate **19d**	6β-OH 3β-OAc **26a**	6β,19-O 3β-OAc **27a**	19-OH 3β-OAc **28b**	19-OH 3β-OH **29b**	25-Keto 3β-OAc **30**
H-1α	1.133	1.049	1.141	1.265	1.203	1.133	1.763†	1.993	1.144	1.093	1.131
H-1β	1.859†	1.800	1.858†	1.966	1.879†	1.869	1.584†	1.695	1.954	1.935	1.856†
H-2α	1.858†	1.716	1.865†	1.985	1.879†	1.899	1.962	1.648	1.871	1.853	1.856†
H-2β	1.587	1.531	1.590	1.683	1.605	1.581	1.635†	1.512	1.494	1.405	1.583
H-3α	4.604	3.487	4.609	4.716	4.675	4.602	5.475	5.197	4.645	3.578	4.602
H-4α	2.327†	2.170	2.339†	2.548	2.356†	2.339†	2.193	2.320	2.421	2.386	2.322†
H-4β	2.308†	2.261	2.321†	2.468	2.356†	2.319†	2.505	2.268	2.267	2.192	2.305†
H-6(α)	5.375	5.315	5.397	5.703	5.585	5.240	4.181	4.055	5.777	5.752	5.372
H-7α	1.541	1.593	1.621†	–	–	5.034	2.243	1.517	1.544	1.533	1.534
H-7β	1.971	1.952	2.010	–	4.963	–	1.667	1.993	2.031	2.033	1.964
H-8β	1.452	1.465	1.662†	2.232	1.594	1.691	1.755	1.609	1.820	1.836	1.45
H-9α	0.953	0.975	1.037	1.529†	1.370†	1.131	1.577	1.662	0.924	0.906	0.945
H-11α	1.511†	1.507†	1.609†	1.564†	1.535†	1.556†	1.432	1.142	1.547†	1.549†	1.500†
H-11β	1.455†	1.424†	1.537†	1.54	1.478†	1.483†	1.301†	1.396	1.625†	1.633†	1.446†
H-12α	1.178	1.283	1.405	1.142	1.192	1.156	1.201†	1.232	1.170	1.161	1.161
H-12β	2.005	1.979	1.776	2.032	2.004	2.015	1.992	1.993	2.040	2.042	1.997
H-14α	1.012†	1.386	1.329	1.343†	1.342†	1.150	1.165†	1.220	0.922	0.912	0.992
H-15α	1.597	1.529†	2.050	2.405	1.430†	1.445	1.574	1.503	1.571	1.569	1.575
H-15β	1.084†	1.620†	1.836	1.245†	1.074	1.285†	1.064†	1.076	1.091	1.093	1.066
H-16α	1.819	–	(5.286)	1.894	1.828	1.807	1.838	1.836	1.810	1.806	1.818
H-16β	1.255	–	–	1.272†	1.263	1.237†	1.257	1.243	1.256	1.258	1.235
H-17α	1.101	1.022	–	1.082	1.137	1.055	1.126	1.124	1.093	1.087	1.106
H-18	0.684	0.697	0.780	0.683	0.669	0.693	0.678	0.693	0.736	0.739	0.673
H-19*R*	1.020	0.992	1.058	1.209	1.013	1.084	1.320	3.919	3.835	3.825	1.016
H-19*S*	–	–	–	–	–	–	–	3.743	3.621	3.609	–
H-20	1.418†	1.539	2.081	1.379	1.385	1.363†	1.368	1.366	1.420†	1.422†	1.393
H-21	0.940	0.941	0.991	0.922	0.926	0.913	0.906	0.894	0.937	0.938	0.932
H-22*R*	1.417†	1.592	1.470*	1.355†	1.345†	1.332†	1.332†	1.326†	1.413†	1.412†	1.342
H-22*S*	1.093	1.147	1.338†*	1.037	1.027	1.018	0.994	0.988	1.089	1.090†	1.039
H-23*R*	1.434†	1.256†	1.232†*	1.22	1.225†	1.218†	1.331†	1.326†	1.431	1.432	1.442
H-23*S*	1.633	1.420†	1.33*	1.33	1.34	1.326†	1.14	1.13	1.628	1.627	1.652
H-24*R*	1.971†	1.350†	1.354†	1.311†	1.303†	1.304†	1.10	1.10	1.967†	1.966	2.361
H-24*S*	2.044†	1.075†	1.118	1.127†	1.125†	1.123†	1.13	1.13	2.043†	2.042	2.406
H-25	–	1.586	1.761†	1.769	1.769	1.766	1.516	1.512	–	–	–
H-26	–	3.355	3.838	3.846	3.840	3.842	0.861	0.859	–	–	2.131
H-26	–	3.436	3.935	3.938	3.945	3.939	–	–	–	–	–
H-27	–	0.864	0.911	0.915	0.919	0.914	0.866	0.863	–	–	–
Other	2.032	[c]	2.034[d]	2.052[d]	2.056[d]	2.021[d]	2.034	2.030	2.031	–	2.030

[a] Data obtained at 500 MHz in 5–15 mM $CDCl_3$ solution at 25°C and referenced to $Si(CH_3)_4$.

[b] 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. An asterisk indicates that stereochemical assignments may be interchanged.

[c] TMDMS signals: δ_H 0.892 (s), 0.889 (s), 0.058 (s), 0.036 (s).

[d] Additional acetate signals: δ_H 2.051 (**15**); 2.057 (**16d**); 2.043, 2.057 (**18d**); 2.030, 2.056 (**19d**).

Table 2
^{13}C NMR chemical shifts of F_7-cholesterol acetate (**2b**), 26-hydroxy derivatives (**11**, **15**, **16d**, **18d**, **19d**), synthetic precursors (**26a**, **27a**, **28b**) to 19-hydroxysterols (**29b**), and 27-norketone **30**[a,b]

		26-Hydroxy derivatives									
	3β-OAc **2b**	16α-OH TBDMS **11**	Δ^{16} Acetate **15**	7-keto Acetate **16d**	7α-OAc Acetate **18d**	7β-OAc Acetate **19d**	6β-OH 3β-OAc **26a**	6β,19-O 3β-OAc **27a**	19-OH 3β-OAc **28b**	19-OH 3β-OH **29b**	25-keto 3β-OAc **30**
C-1	36.96	37.25	36.92	35.97	36.48	36.48	35.08	32.80	33.07	33.32	36.94
C-2	27.73	32.03	27.74	27.30	27.47	27.57	26.32	23.24	28.05	31.91	27.73
C-3	73.93	72.55	73.92	72.16	73.12	73.18	72.13	69.99	73.36	71.32	73.94
C-4	38.08	42.77	38.12	37.69	37.80	37.48	38.39	41.31	38.16	42.25	38.08
C-5	139.63	141.51	139.87	163.85	146.47	144.17	86.71	74.54	134.48	135.50	139.62
C-6	122.56	120.98	122.54	126.65	120.82	122.21	75.72	82.30	128.26	127.33	122.59
C-7	31.83	31.83	31.59	201.92	68.24	75.49	34.54	26.86	31.16	31.17	31.84
C-8	31.81	31.19	30.53	45.35	35.72	36.42	30.54	33.25	33.32	33.37	31.81
C-9	49.94	49.99	50.65	49.72	43.00	48.02	47.38	48.64	50.21	50.29	49.94
C-10	36.55	36.57	36.80	38.26	37.24	36.45	40.31	45.81	41.55	41.47	36.55
C-11	20.98	20.73	20.75	21.11	20.63	20.99	21.27	22.64	21.69	21.73	20.97
C-12	39.65	39.85	35.02	38.59	38.99	39.27	39.62	39.70	39.91	39.96	39.64
C-13	42.33	44.12	46.89	43.07	42.23	42.79	42.66	43.13	42.53	42.54	42.28
C-14	56.59	53.65	57.28	49.88	49.14	55.41	55.68	54.32	57.46	57.55	56.61
C-15	24.21	36.86	30.95	26.24	24.02	25.09	24.03	23.45	24.00	24.01	24.23
C-16	28.20	[c]	[c]	28.53	28.13	28.35	28.17	28.25	28.19	28.20	28.18
C-17	55.86	66.96	160.52	54.68	55.83	55.30	56.06	55.97	55.79	55.80	55.75
C-18	11.82	13.40	16.16	11.93	11.43	11.74	12.16	12.38	12.17	12.18	11.81
C-19	19.29	19.41	19.21	17.21	18.13	18.95	18.01	67.48	62.68	62.69	19.28
C-20	35.53	33.92	32.17	35.60	35.64	35.57	35.74	35.71	35.49	35.48	35.61
C-21	18.46	18.88	21.87	18.76	18.66	18.65	18.64	18.58	18.45	18.45	18.57
C-22	35.99	35.92	36.65	35.94	36.00	35.92	36.10	36.09	35.97	35.97	35.40
C-23	18.09	24.03	24.73	23.20	23.25	23.17	23.78	23.75	18.06	18.04	20.38
C-24	29.38	33.42	33.50	33.63	33.72	33.63	39.47	39.47	29.37	29.37	44.25
C-25	[c]	35.75	32.46	32.41	32.49	32.41	27.99	27.99	91.81	91.81	209.43
C-26	121.11	68.55	69.47	69.55	69.57	69.56	22.54	22.55	121.10	121.08	29.87
C-27	121.11	16.62	16.86	16.72	16.78	16.72	22.80	22.80	121.10	121.08	–
Other	21.43	−4.61	21.41	21.24	21.34	21.35	21.36	21.31	21.37	–	21.44
	170.54	18.26	170.52	170.27	170.41	170.34	170.48	170.36	170.53	–	170.53
	–	25.93	20.96	20.97	20.97	20.98	–	–	–	–	–
	–	−5.33	171.28	171.31	171.29	171.31	–	–	–	–	–
	–	18.36	–	–	21.34	21.63	–	–	–	–	–
	–	25.96	–	–	170.79	171.09	–	–	–	–	–

[a] Chemical shifts (± 0.03 ppm) obtained at 125 MHz at 22°C in 20–120 mM $CDCl_3$ solution and referenced to the $CDCl_3$ signal at 77.0 ppm.

[b] Coupling patterns for F_7 sterols **2b**, **28b**, and **29b**: C-23, broad ($W_{1/2}$ 9 Hz); C-24, d, 20 Hz; C-25, d of quintet, 202, 32 Hz; C-26 and C-27, qd, 286, 28 Hz.

[c] Not determined because of insufficient sensitivity due to splittings from coupling to ^2H or ^{19}F.

Similar acetylation of **1c** (500 mg) gave **2c** (510 mg, 92% yield, 98% purity): m.p., 111–113°C; single component by TLC (R_f 0.60, SS-1); high-resolution MS, calcd. for $C_{27}H_{38}D_6$ (M − 60), 374.3820, found 374.3796; MS, 374*† (100), 359*† (12), 283 (1), 266† (5, $C_{19}H_{26}D_6$), 255*† (9), 253† (10, $C_{18}H_{25}D_6$), 213† (8, $C_{16}H_{21}$), 211† (2, $C_{15}H_{19}D_6$), 147† (21, $C_{11}H_{15}$), 105† (19, C_8H_9); IR, 2964, 2937, 2904, 2862, 2827, 2216, 2121, 2065, 1730, 1465, 1440, 1369, 1249, 1199, 1134, 1118, 1039 cm^{-1}; NMR, δ_H 5.375 (m), 4.603 (m), 2.32 (m), 2.31 (m), 2.033 (s, 3H), 1.018 (d, 0.6 Hz, 3H),

0.914 (d, 6.6 Hz, 3H), 0.676 (d, 0.5 Hz, 3H), δ_C 170.54, 139.62, 122.63, 73.97, 56.65, 56.09, 49.99, 42.28, 39.69, 39.36, 38.09, 36.96, 36.56, 36.16, 35.78, 31.87, 31.82, 28.21, 27.74, 27.51, 24.26, 23.80, 21.44, 21.00, 19.29, 18.69, 11.83 (no signals observed for C-26, C-27).

*2.3. 25,26,26,26,27,27,27-Heptafluorocholest-5-ene-3β,4β-diol (**3b**), cholest-5-ene-3β,4β-diol (**3a**), and [26,26,26,27,27,27-$^2H_6$]cholest-5-ene-3β,4β-diol (**3c**)*

To a solution of acetate **2b** (80 mg, 0.14 mmol) in toluene (20 ml) were added selenium dioxide (15.4 mg, 0.14 mmol) and potassium phosphate monobasic (19 mg, 0.14 mmol). The reaction mixture was refluxed for 4 h, followed by removal of the inorganic salts by filtration. The filtrate was dried over anhydrous sodium sulfate and evaporated to a light yellow solid that was subjected to MPLC (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 5:95). The crude acetate product (30 mg, ~90% purity) was dissolved in methanol (20 ml), sodium carbonate (10 mg, 0.1 mmol) was added, and the resulting solution was stirred at room temperature overnight. TLC analysis showed a major product at R_f 0.53 (SS-5). After evaporation of methanol, the resulting residue was dissolved in dichloromethane (100 ml), washed with brine (10 ml), dried over anhydrous sodium sulfate, and evaporated to a white solid. MPLC (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 15:85) followed by evaporation of fractions 49–56 furnished **3b** as a white solid (26 mg, 34% yield; 98% purity): m.p., 178.5–179.5°C; single component by TLC (R_f 0.53, SS-5); high-resolution MS, calcd. for $C_{27}H_{39}O_2F_7$, 528.2838, found 528.2834; MS, 528† (24, M^+), 513*† (17), 510*† (63), 495*† (15), 484† (41, $M-C_2H_4O$), 471† (15, $M-C_3H_5O$), 468† (9, $M-C_2H_4O_2$), 453† (9, $M-C_3H_7O_2$), 413† (8, $M-C_6H_{11}O_2$), 401† (11, $M-C_7H_{11}O_2$), 387† (10, $M-C_8H_{13}O_2$), 373† (17, $M-C_9H_{15}O_2$), 271*† (12), 253*† (5), 239† (13, SC), 229† (24, $C_{16}H_{21}O$), 124† (40, $C_8H_{12}O$), 105† (55, C_8H_9), 55 (100, C_4H_7); IR, 3418, 2942, 2899, 2872, 1468, 1318, 1223, 1159, 1074, 966 cm^{-1}; ^{1}H NMR, Table 3; ^{13}C NMR, Table 4; ^{19}F NMR, δ_F −184.16 (dd of septet, 21.2, 19.9, 6.6 Hz), −76.74 and −76.95 (A_3B_3 portion of A_3B_3X system (Swaminathan et al., 1993)).

Similar treatment of **2a** (0.43 g) gave **3a** as a white solid (0.16 g, 40% yield; > 99% purity): m.p., 176–177°C (lit. 176–177°C (Rosenheim and Starling, 1937), 175–175.5°C (Breuer, 1995)); single component by TLC (R_f 0.57, SS-5); MS, 402 (46, M^+), 387* (39), 384* (100), 369* (30), 366* (23), 358 (84), 345 (30), 342 (12), 327 (14), 287 (13), 275 (18), 271* (29), 261 (17), 253 (9), 247 (33), 229 (49), 124 (51), 105 (56); ^{1}H and ^{13}C NMR data identical (±0.001 ppm for ^{1}H, ±0.03 ppm for ^{13}C) to those of **3c** except for the occurrence of signals at δ_H 1.517 (nonet, 6.6 Hz), 0.867 (d, 6.6 Hz, 3H), 0.862 (d, 6.6 Hz, 3H), δ_C 39.48 (replacing 39.35), 27.99 (replacing 27.50), 22.80, 22.54.

Similar treatment of **2c** (52 mg) gave **3c** as a white solid (20 mg, 41% yield; ≥ 99% purity): m.p., 174–175°C; single component by TLC (R_f 0.57, SS-5); high-resolution MS, calcd. for $C_{27}H_{40}D_6O_2$, 408.3874, found 408.3877; MS, 408† (22, M^+), 393*† (16), 390*† (100), 375*† (18), 364† (40, $M-C_2H_4O$), 351† (12, $M-C_3H_5O$), 348 (15), 333† (8, $M-C_3H_7O_2$), 293† (5, $M-C_6H_{11}O_2$), 281† (13, $M-C_7H_{11}O_2$), 271*† (22), 267† (24, $M-C_8H_{13}O_2$), 253† (32, $M-C_9H_{15}O_2$ and $M-2H_2O$–SC), 229† (55, $C_{16}H_{21}O$), 124† (73, $C_8H_{12}O$), 105† (32, C_8H_9); IR, 3416, 3386, 2937, 2904, 2866, 2820, 2204, 1456, 1381, 1363, 1070, 1094, 964 cm^{-1}; NMR, δ_H 5.681 (dd, 4.6, 1.8 Hz), 4.139 (dd, 3.5, 1.2 Hz), 3.562 (dt, 11.8, 4.2 Hz), 2.087 (m), 2.014 (dt, 12.7, 3.6 Hz), 1.908 (dt, 13.3, 3.5 Hz), 1.649 (ddtd, 12.6, 4.8, 3.5, 1.3 Hz), 1.182 (d, 0.7 Hz, 3H), 0.914 (d, 6.7 Hz, 3H), 0.680 (d, 0.5 Hz, 3H), δ_D 0.83 (s), δ_C 142.72, 128.78, 77.25, 72.45, 56.88, 56.06, 50.15, 42.28, 39.65, 39.35, 36.89, 36.16, 35.97, 35.76, 32.06, 31.78, 28.20, 27.50, 25.36, 24.23, 23.79, 21.03, 20.51, 18.68, 11.84 (no signals observed for C-26, C-27).

*2.4. 5,6α-Epoxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-3β-ol acetate (**4b**), 5,6α-epoxy-5α-cholestan-3β-ol acetate (**4a**), and [26,26,26,27,27,27-$^2H_6$]5,6α-epoxy-5α-cholestan-3β-ol acetate (**4c**)*

To a solution of acetate **2b** (70 mg, 0.13 mmol)

Table 3
^{1}H NMR chemical shifts of sterols oxygenated in rings A or B[a,b]

	4β-OH 3β-OH **3b**	α-Epox 3β-OAc **4b**	α-Epox 3β-OH **5b**	β-Epox 3β-OAc **6b**	β-Epox 3β-OH **7b**	5α,6β-OH 3β-OH **8b**	7-Keto 3β-OAc **16b**	7-Keto 3β-OH **17b**	7α-OAc 3β-OAc **18b**	7β-OAc 3β-OAc **19b**	7α-OH 3β-OH **20b**	7β-OH 3β-OH **21b**
H-1α	1.076	1.423	1.367	1.320	1.259	1.54	1.265	1.211	1.204	1.134	1.121	1.059
H-1β	1.831	1.694	1.690	1.963	1.967	1.422	1.967†	1.955†	1.880†	1.870	1.868†	1.854†
H-2α	1.651	1.995	1.921	1.830	1.811	1.867	1.987†	1.945†	1.880†	1.901	1.858†	1.856†
H-2β	1.909	1.637	1.606	1.497	1.411	1.52	1.685	1.624	1.606	1.583	1.524	1.520
H-3α	3.563	4.949	3.914	4.768	3.700	4.098	4.717	3.680	4.676†	4.603†	3.591	3.553
H-4α	4.140	1.328	1.295	1.437	1.430	1.614	2.551	2.511	2.358†	2.343†	2.349	2.338
H-4β	–	2.158	2.073	2.113	2.041	2.088	2.469	2.401	2.358†	2.319†	2.289	2.258
H-6	5.681	2.892	2.902	3.076	3.061	3.545	5.707	5.696	5.585	5.239	5.609	5.294
H-7α	1.57	1.498	1.489	1.208	1.201	1.642†	–	–	–	5.038	–	3.845
H-7β	2.090	1.908	1.914	2.074	2.077	1.593†	–	–	4.966	–	3.852	–
H-8β	1.55	1.368	1.376	1.477	1.489	1.732	2.235	2.243	1.600	1.696	1.477†	1.399
H-9α	0.894	1.284†	1.257†	0.626	0.609	1.242	1.54	1.506†	1.374	1.135	1.224	1.038
H-11α	1.472†	1.377	1.382	1.372†	1.378†	1.400†	1.59	1.59	1.545†	1.566†	1.549†	1.543†
H-11β	1.447†	1.243†	1.257†	1.404†	1.408†	1.337†	1.56	1.57	1.485†	1.487†	1.494†	1.468†
H-12α	1.164	1.125	1.119	1.075	1.064	1.168	1.156	1.143	1.206	1.171	1.183	1.154
H-12β	2.010	1.942	1.944	1.952	1.954	1.994	2.030	2.030	2.004	2.014	2.001	2.017
H-14α	0.998†	0.969†	0.961†	0.888	0.877	1.094†	1.356†	1.348	1.353	1.166	1.449†	1.164
H-15α	1.599	1.576	1.577	1.598	1.594	1.589	2.424	2.427	1.449	1.462	1.734	1.832†
H-15β	1.096†	1.015†	1.020†	1.076	1.081	1.092†	1.264†	1.254†	1.092	1.299†	1.157	1.450
H-16α	1.821	1.804	1.802	1.801	1.797	1.813	1.889	1.887	1.822	1.802	1.886	1.872†
H-16β	1.265	1.220	1.223	1.245	1.246	1.253	1.270†	1.272†	1.265	1.238†	1.290	1.307
H-17α	1.096	1.059	1.056	1.064	1.058	1.102	1.092	1.088	1.150	1.066	1.170	1.087
H-18	0.688	0.616	0.618	0.645	0.647	0.687	0.690	0.691	0.677	0.701	0.694	0.703
H-19	1.184	1.074	1.061	1.004	0.996	1.184	1.212	1.202	1.015	1.086	0.998	1.054
H-20	1.421†	1.381†	1.385†	1.404†	1.405†	1.412†	1.419†	1.423†	1.422†	1.405†	1.437†	1.429†
H-21	0.940	0.912	0.912	0.916	0.916	0.930	0.948	0.948	0.951	0.939	0.953	0.949
H-22*R*	1.416†	1.399†	1.397†	1.400†	1.401†	1.41	1.437†	1.437†	1.424†	1.411†	1.420†	1.433†
H-22*S*	1.094	1.069†	1.069	1.086†	1.085†	1.086	1.106	1.107	1.091	1.086†	1.114	1.106
H-23*R*	1.436†	1.416†	1.418†	1.423	1.424	1.430†	1.431†	1.435†	1.439†	1.429†	1.436†	1.438†
H-23*S*	1.631	1.624	1.623	1.618	1.617	1.627†	1.636	1.635	1.642	1.631	1.632	1.637
H-24*R*	1.971†	1.963†	1.964†	1.963†	1.962†	1.966†	1.975†	1.976†	1.974†	1.971†	1.974†	1.976†
H-24*S*	2.044†	2.034†	2.035†	2.038†	2.038†	2.042†	2.046†	2.047†	2.045†	2.045†	2.048†	2.050†
Acetate	–	2.013	–	2.029	–	–	2.052	–	2.038	2.023	–	–
	–	–	–	–	–	–	–	–	2.046	2.030	–	–

[a] See footnote a of Table 1.
[b] See footnote b of Table 1.

Table 4
^{13}C NMR chemical shifts of 25,26,26,26,27,27,27-heptafluoro derivatives of sterols oxygenated in rings A or B[a,b,c]

	4β-OH 3β-OH **3b**	α-Epox 3β-OAc **4b**	α-Epox 3β-OH **5b**	β-Epox 3β-OAc **6b**	β-Epox 3β-OH **7b**	5α,6β-OH 3β-OH **8b**	7-Keto 3β-OAc **16b**	7-Keto 3β-OH **17b**	7α-OAc 3β-OAc **18b**	7β-OAc 3β-OAc **19b**	7α-OH 3β-OH **20b**	7β-OH 3β-OH **21b**
C-1	36.88	32.09	32.36	36.65	37.21	32.34	35.96	36.30	36.47	36.48	36.97	36.93
C-2	25.34	27.17	31.04	27.16	31.00	30.82	27.30	31.11	27.45	27.57	31.31	31.55
C-3	72.45	71.34	68.68	71.29	69.39	67.61	72.15	70.43	73.10	73.17	71.27	71.40
C-4	77.23	36.07	39.82	37.95	42.18	40.72	37.71	41.78	37.79	37.48	41.97	41.71
C-5	142.71	65.17	65.68	62.50	62.92	76.07	163.90	165.30	146.48	144.19	146.25	143.51
C-6	128.70	59.11	59.24	63.53	63.67	76.04	126.65	126.00	120.78	122.18	123.79	125.46
C-7	32.02	28.68	28.75	32.39	32.55	34.47	201.83	202.26	68.19	75.44	65.29	73.30
C-8	31.77	29.81	29.85	29.69	29.74	30.17	45.33	45.32	35.69	36.42	37.45	40.88
C-9	50.10	42.35	42.48	50.90	51.25	45.79	49.70	49.82	42.96	48.00	42.19	48.20
C-10	35.96	34.95	34.82	34.98	34.82	38.27	38.28	38.25	37.23	36.45	37.36	36.43
C-11	20.49	20.53	20.59	21.87	21.94	21.13	21.10	21.15	20.61	20.98	20.65	21.06
C-12	39.61	39.29	39.34	39.69	39.75	39.87	38.58	38.61	38.95	39.25	39.11	39.52
C-13	42.33	42.34	42.35	42.28	42.30	42.76	43.11	43.09	42.28	42.83	42.15	42.96
C-14	56.81	56.67	56.76	56.06	56.12	55.85	49.86	49.86	49.11	55.36	49.37	55.90
C-15	24.18	23.97	23.97	24.10	24.11	24.07	26.21	26.22	23.99	25.04	24.21	26.34
C-16	28.18	28.05	28.05	28.10	28.10	28.18	28.54	28.53	28.12	28.34	28.25	28.55
C-17	55.80	55.62	55.62	55.86	55.88	55.94	54.53	54.53	55.66	55.15	55.58	55.21
C-18	11.82	11.81	11.82	11.71	11.72	12.13	11.93	11.92	11.43	11.73	11.60	11.82
C-19	21.01	15.81	15.89	17.00	17.03	16.86	17.22	17.26	18.14	18.94	18.22	19.16
C-20	35.50	35.52	35.51	35.43	35.42	35.51	35.48	35.46	35.52	35.45	35.52	35.50
C-21	18.46	18.38	18.38	18.42	18.42	18.43	18.60	18.59	18.47	18.49	18.49	18.55
C-22	35.98	35.93	35.94	35.92	35.93	35.96	35.96	35.97	35.94	35.94	35.99	36.02
C-23	18.06	18.16	18.14	18.00	18.00	18.09	18.12	18.10	18.15	18.14	18.00	18.10
C-24	29.38	29.34	29.35	29.36	29.36	29.38	29.35	29.34	29.33	29.34	29.40	29.40
C-25	c	91.80	91.81	c	91.81	c	c	91.79	c	91.79	c	c
C-26	121.10	121.09	121.08	121.09	121.09	c	c	121.09	121.10	121.09	121.10	121.13
C-27	121.10	121.09	121.08	121.09	121.09	c	c	121.09	121.10	121.09	121.10	121.13
Acetate	–	21.31	–	21.30	–		21.25	–	21.36[d]	21.34[d]	–	–
	–	170.23	–	170.56	–		170.29	–	170.45[d]	170.36[d]	–	–

[a] See footnote a of Table 2.
[b] See footnote b of Table 2.
[c] See footnote c of Table 2.
[d] Additional acetate signals: δ_C 21.36, 170.81 (**18b**), 21.62, 171.10 (**19b**).

in dichloromethane (15 ml) were added *m*-chloroperbenzoic acid (26 mg, 85% purity, 0.13 mmol) and sodium bicarbonate (17 mg, 0.20 mmol). The reaction mixture was stirred at room temperature for 36 h, followed by dilution with dichloromethane (20 ml), washing with 10% aqueous sodium hydroxide solution (2 × 10 ml), water (10 ml), and brine (10 ml), and drying over anhydrous sodium sulfate. Evaporation gave a white solid that was purified by MPLC on alumina–$AgNO_3$ (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 3:97). Evaporation of fractions 60–65 gave **4b** (40 mg, 56% yield, 98% purity, containing 0.2% **6b**): m.p., 154.5–155.5°C; single component by TLC (R_f 0.24, SS-1); high-resolution MS, calcd. for $C_{29}H_{41}O_3F_7$, 570.2944, found 570.2926; MS, Table 5; IR, 2955, 2874, 2852, 1738, 1470, 1381, 1311, 1244, 1223, 1157, 1132, 1042, 939 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4.

Similar treatment of **2a** (200 mg) gave **4a** (146 mg, 70% yield, ~98% purity, containing 0.2% **6a**): m.p., 98–100°C (lit. 97–98°C (Kudo et al., 1989); 101°C (Fieser and Fieser, 1959)); single component by TLC (R_f 0.31, SS-1); MS, Table 5; ^1H and ^{13}C NMR data identical ($\pm$0.001 ppm for ^1H, $\pm$0.05 ppm for ^{13}C) to those of **4c** except for the occurrence of signals at δ_H 1.510 (nonet, 6.6 Hz), 0.862 (d, 6.6 Hz, 3H), 0.857 (d, 6.6 Hz, 3H), δ_C 39.45 (replacing 39.32), 27.97 (replacing 27.48), 22.80, 22.53.

Similar treatment of **2c** (25 mg) gave **4c** (20 mg, 77% yield, ~99% purity, containing 0.5% **6c**): m.p., 94–96°C; single component by TLC (R_f 0.31, SS-1); high-resolution MS, calcd. for $C_{29}H_{42}D_6O_3$, 450.3980, found 450.3981; MS, Table 5; IR, 2943, 2868, 2212, 1736, 1467, 1377, 1240, 1028 cm^{-1}; NMR, δ_H (50 mM solution) 4.949, (tt, 11.5, 4.9 Hz), 2.888 (d, 4.3 Hz), 2.157 (dd, 12.7, 11.6 Hz), 2.013 (s, 3H), 1.904 (ddd, 15.6, 11.9, 4.5 Hz), 1.494 (dd, 15.5, 9.8 Hz), 1.324 (ddd, 12.7, 5.0, 2.2 Hz), 1.073 (d, 0.5 Hz, 3H), 0.886 (d, 6.6 Hz, 3H), 0.607 (s, 3H); δ_C 170.21, 71.37, 65.16, 59.14, 56.72, 55.79, 42.38, 42.28, 39.32, 39.32, 36.09, 36.09, 35.74, 34.95, 32.08, 29.81, 28.72, 28.05, 27.48, 27.18, 24.01, 23.80, 21.33, 20.54, 18.60, 15.82, 11.82 (no signals observed for C-26, C-27).

*2.5. 5,6α-Epoxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-3β-ol (**5b**), 5,6α-epoxy-5α-cholestan-3β-ol (**5a**), and [26,26,26,27,27,27-$^2H_6$] 5,6α-epoxy-5α-cholestan-3β-ol (**5c**)*

To a solution of **4b** (30 mg, 0.053 mmol) in methanol (10 ml) was added sodium carbonate (10 mg, 0.1 mmol), and the reaction mixture was stirred at room temperature overnight. TLC analysis showed the disappearance of **4b** and the formation of a polar compound at R_f 0.32 (SS-4). The solvent was evaporated to a residue that was dissolved in dichloromethane, washed with brine (10 ml), and dried over anhydrous sodium sulfate. Evaporation gave a white solid that was filtered through silica gel (30 × 5 mm i.d. column; elution with ethyl acetate–hexane 15:85). Evaporation gave **5b** as a white solid (25 mg, 90% yield, 98% purity): m.p., 169–170°C; single component by TLC (R_f 0.32, SS-4); high-resolution MS, calcd. for $C_{27}H_{39}O_2F_7$, 528.2838, found 528.2827; MS, Table 5; IR, 3478, 2938, 2870, 1468, 1314, 1221, 1161, 1061, 966 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4; ^{19}F NMR, δ_F ca. −184 (dd of septet, 21.1, 19.9, 6.7 Hz), −76.74 and −76.94 (A_3B_3 portion of A_3B_3X system (Swaminathan et al., 1993)).

Similar treatment of **4a** (50 mg) gave **5a** as a white solid (41 mg, 91% yield, 99% purity): m.p., 140–141.5°C (lit. 142.5°C (Fieser and Fieser, 1959)); single component by TLC (R_f 0.35, SS-4); MS, Table 5; ^1H and ^{13}C NMR data identical ($\pm$0.004 ppm for ^1H, $\pm$0.06 ppm for ^{13}C) to those of **5c** except for the occurrence of signals at δ_H 1.510 (nonet, 6.6 Hz), 0.862 (d, 6.6 Hz, 3H), 0.857 (d, 6.6 Hz, 3H), δ_C 27.97 (replacing 27.49), 22.80, 22.53.

Similar treatment of **4c** (15 mg) gave **5c** as a white solid (12 mg, 88% yield, 99% purity): m.p., 139–140°C; single component by TLC (R_f 0.35, SS-4); high-resolution MS, calcd. for $C_{27}H_{40}D_6O_2$, 408.3874, found 408.3888; MS, Table 5; IR, 3450, 2934, 2866, 2214, 1466, 1443, 1371, 1337, 1099, 1063, 1042 cm^{-1}; NMR, δ_H (50 mM solution) 3.908 (tt, 11.3, 4.8 Hz), 2.899 (d, 4.3 Hz), 2.071 (dd, 12.7, 11.3 Hz), 1.910 (ddd, 15.6, 7.5, 4.5 Hz), 1.689 (ddd, 13.3, 4.2, 2.9 Hz), 1.603 (dddd, 13.5, 12.7, 11.3, 4.3 Hz), 1.484 (dd, 15.4, 9.8 Hz), 1.290

Table 5
Comparison of mass spectral data for α and β epoxides **5a** and **7a**, their 25,26,26,26,27,27,27-heptafluoro (F_7) derivatives **5b** and **7b**, their 26,26,26,27,27,27-hexadeuterio (d_6) derivatives **5c** and **7c**, and acetate derivatives of the six epoxides[a]

	3β-Hydroxy ($R = H$)						3β-Acetates ($R = Ac$)					
	F_7-α **5b**	F_7-β **7b**	α **5a**	β **7a**	d_6-α **5c**	d_6-β **7c**	F_7-α **4b**	F_7-β **6b**	α **4a**	β **6a**	d_6-α **4c**	d_6-β **6c**
M+	528 (65)	(56)	402 (85)	(67)	408 (61)	(73)	570 (5)	(5)	444 (10)	(8)	450 (4)	(4)
M−Me	513 (1)	(6)	387 (1)	(20)	393 (1)	(9)	555 (1)	(1)	429 (2)	(4)	435 (0)	(1)
$M-H_2O$	510 (49)	(48)	384 (72)	(100)	390 (89)	(95)	552 (3)	(2)	426 (7)	(3)	432 (4)	(1)
$M-H_2O$–Me	495 (18)	(20)	369 (34)	(36)	375 (34)	(37)	537 (2)	(1)	411 (3)	(1)	417 (2)	(1)
M−AcOH	–	–	–	–	–	–	510 (100)	(100)	384 (100)	(100)	390 (100)	(100)
M−AcOH–Me	–	–	–	–	–	–	495 (25)	(30)	369 (26)	(21)	375 (20)	(20)
$M-ROH-H_2O$	492 (14)	(21)	366 (17)	(27)	372 (27)	(35)	492 (43)	(34)	366 (46)	(19)	372 (37)	(18)
M−ROH–CO	482 (4)	(16)	356 (6)	(24)	362 (12)	(44)	482 (15)	(42)	356 (13)	(32)	362 (6)	(36)
M−ROH–CHO	481 (5)	(17)	355 (5)	(20)	361 (12)	(56)	481 (7)	(29)	355 (5)	(14)	361 (14)	(23)
$M-ROH-H_2O$–Me	477 (10)	(5)	351 (17)	(9)	357 (11)	(8)	477 (15)	(8)	351 (16)	(7)	357 (12)	(5)
$M-ROH-C_2H_4O$	466 (3)	(2)	340 (4)	(1)	346 (3)	(2)	466 (9)	(4)	340 (13)	(5)	346 (10)	(3)
Ion B_1	401 (8)	(9)	275 (12)	(9)	281 (7)	(13)	401 (4)	(6)	275 (5)	(5)	281 (6)	(7)
Ion B_2	387 (7)	(5)	261 (25)	(11)	267 (10)	(10)	387 (5)	(5)	261 (5)	(4)	267 (5)	(4)
Ion B_3	373 (15)	(15)	247 (24)	(29)	253 (20)	(27)	373 (12)	(14)	247 (9)	(13)	253 (25)	(20)
Ion C_1	333 (5)	(4)	207 (5)	(2)	213 (9)	(11)	333 (4)	(3)	207 (3)	(1)	213 (7)	(6)
Ion C_2	319 (9)	(6)	193 (14)	(7)	199 (11)	(10)	319 (7)	(4)	193 (5)	(3)	199 (4)	(7)
M−SC	289 (3)	(2)	289 (10)	(29)	289 (4)	(10)	331 (4)	(4)	331 (8)	(13)	331 (2)	(2)
M−SC–ROH	271 (8)	(6)	271 (22)	(24)	271 (15)	(13)	271 (7)	(4)	271 (13)	(10)	271 (11)	(8)
$M-SC-ROH-H_2O$	253 (5)	(4)	253 (11)	(11)	253 (20)	(27)	253 (12)	(6)	253 (20)	(10)	253 (25)	(20)
SC	239 (16)	(17)	[b]		[b]		239 (6)	(5)	[b]		[b]	
$C_{16}H_{22}O$	230 (3)	(4)	230 (7)	(5)	230 (6)	(12)	230 (3)	(2)	230 (3)	(4)	230 (4)	(4)
$C_{16}H_{21}O$	229 (13)	(11)	229 (19)	(25)	229 (19)	(24)	229 (8)	(7)	229 (13)	(10)	229 (11)	(12)
$C_{16}H_{19}$	211 (22)	(6)	211 (20)	(24)	211 (14)	(13)	211 (13)	(10)	211 (16)	(15)	211 (15)	(7)
C_9H_{12}	120 (40)	(16)	120 (65)	(18)	120 (30)	(25)	120 (26)	(8)	120 (30)	(19)	120 (28)	(12)
C_8H_{11}	107 (49)	(45)	107 (71)	(50)	107 (60)	(72)	107 (33)	(24)	107 (32)	(39)	107 (26)	(25)
C_4H_7	55 (100)	(100)	55 (100)	(34)	55 (100)	(100)	55 (53)	(30)	55 (45)	(49)	55 (22)	(25)

[a] Mass spectral data are given in the form m/z (relative abundance of α isomer) (relative abundance of β isomer). Ions B_1, B_2, B_3, C_1, and C_2 are defined in Fig. 7.

[b] Exact masses of ions at m/z 113 and 119 did not correspond to C_8H_{17} or $C_8H_{11}D_6$.

(ddd, 12.8, 4.9, 2.3 Hz), 1.059 (d, 0.6 Hz, 3H), 0.886 (d, 6.6 Hz, 3H), 0.609 (d, 0.5 Hz, 3H), δ_C (25°C) 68.72, 65.68, 59.29, 56.84, 55.85, 42.54, 42.32, 39.86, 39.40, 39.34, 36.13, 35.74, 34.84, 32.39, 31.08, 29.88, 28.81, 28.06, 27.49, 24.03, 23.82, 20.63, 18.62, 15.91, 11.85 (no signals observed for C-26, C-27).

*2.6. 5,6β-Epoxy-25,26,26,26,27,27,27-heptafluoro-5β-cholestan-3β-ol acetate (**6b**), 5,6β-epoxy-5β-cholestan-3β-ol acetate (**6a**), and [26,26,26,27,27,27-$^2H_6$]5,6β-epoxy-5β-cholestan-3β-ol acetate (**6c**)*

Copper sulfate pentahydrate (0.25 g) and potassium permanganate (0.50 g) were ground together to a fine powder, to which water (40 μl) was added. This paste was transferred to a 15 ml flask containing a solution of **2b** (80 mg, 0.144 mmol) in dichloromethane (10 ml), followed by addition of *tert*-butanol (0.1 ml). The reaction mixture was heated to reflux for 10 min and stirred at room temperature overnight. TLC analysis showed the absence of **2b** and the formation of polar material. The reaction mixture was filtered through a pad of silica gel (230–400 mesh), followed by washing with dichloromethane (50 ml). The filtrate was dried over anhydrous sodium sulfate and evaporated to a white solid, which was subjected to MPLC on alumina–$AgNO_3$ (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 3:97). Evaporation of fractions 25–40 gave **6b** (45 mg, 55% yield, ~97% purity): m.p., 136–137°C; single component by TLC (R_f 0.24, SS-1); high-resolution MS, calcd. for $C_{29}H_{41}O_3F_7$, 570.2944, found 570.2928; MS, Table 5; IR, 2949, 2870, 1738, 1470, 1379, 1312, 1226, 1155, 1032, 966, 939 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4.

Similar treatment of **2a** (200 mg) gave **6a** (165 mg, 80% yield, ~99% purity): m.p., 109.5–110.5°C (lit. 110°C (Marchon and Ramasseul, 1989), 112–113°C (Kudo et al., 1989), 113°C (Fieser and Fieser, 1959)); single component by TLC (R_f 0.31, SS-1); MS, Table 5; ^1H and ^{13}C NMR data identical ($\pm$0.001 ppm for ^1H, $\pm$0.05 ppm for ^{13}C) to those of **6c** except for the occurrence of signals at δ_H 1.511 (nonet, 6.6 Hz), 0.862 (d, 6.6 Hz, 3H), 0.858 (d, 6.6 Hz, 3H), δ_C 39.45 (replacing 39.32), 27.98 (replacing 27.49), 22.79, 22.53.

Similar treatment of **2c** (25 mg) gave **6c** (22 mg, 85% yield, >99% purity): m.p., 108–109°C; single component by TLC (R_f 0.31, SS-1); high-resolution MS, calcd. for $C_{29}H_{42}D_6O_3$, 450.3980, found 450.4003; MS, Table 5; IR, 2932, 2866, 2214, 1730, 1468, 1366, 1248, 1041 cm^{-1}; NMR, δ_H (50 mM solution) 4.767 (ddt, 11.9, 10.3, 4.8 Hz), 3.073 (br dd, 2.7, 0.8 Hz), 2.112 (ddd, ~13, ~12, 0.5 Hz), 2.073 (dtd, 14.4, 3.3, 0.5 Hz), 2.028 (s, 3H), 1.575 (dddd, 12.3, 9.9, 7.3, 3.0 Hz), 1.435 (ddd, 13.1, 5.1, 2.2 Hz), 1.202 (ddd, 14.6, 10.9, 1.1 Hz), 1.002 (s, 3H), 0.889 (d, 6.6 Hz, 3H), 0.637 (s, 3H); δ_C 71.31, 63.57, 62.49, 56.13, 56.13, 50.94, 42.23, 39.73, 39.32, 37.97, 36.64, 36.09, 35.70, 34.98, 32.42, 29.69, 28.12, 27.49, 27.17, 24.14, 23.76, 21.89, 18.64, 17.01, 11.73 (no signals observed for C-26, C-27).

*2.7. 5,6β-Epoxy-25,26,26,26,27,27,27-heptafluoro-5β-cholestan-3β-ol (**7b**), 5,6β-epoxy-5β-cholestan-3β-ol (**7a**), and [26,26,26,27,27,27-$^2H_6$] 5,6β-epoxy-5β-cholestan-3β-ol (**7c**)*

As described above for **4b**, saponification of **6b** (40 mg, 0.070 mmol) in methanol (10 ml) and sodium carbonate (10 mg, 0.1 mmol) gave after workup **7b** as a white solid (34 mg, 92% yield, 98% purity): m.p., 138.5–140°C; single component by TLC (R_f 0.32, SS-4); high-resolution MS, calcd. for $C_{27}H_{39}O_2F_7$, 528.2838, found 528.2841; MS, Table 5; IR, 3439, 3366, 2944, 2870, 1468, 1314, 1221, 1159, 1065, 938 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4; ^{19}F NMR, δ_F −184.18 (dd of septet, 21.2, 19.8, 6.6 Hz), −76.73 and −76.95 (A_3B_3 portion of A_3B_3X system (Swaminathan et al., 1993)).

Similar treatment of **6a** (100 mg) gave **7a** (85 mg, 94% yield, >99% purity): m.p., 129–130°C (lit. 131°C (Baxter and Spring, 1943), 132°C (Fieser and Fieser, 1959)); single component by TLC (R_f 0.35, SS-4); MS, Table 5; ^1H and ^{13}C NMR data identical ($\pm$0.003 ppm for ^1H, $\pm$0.04 ppm for ^{13}C) to those of **7c** except for the occurrence of signals at δ_H 1.510 (nonet, 6.6 Hz), 0.862 (d, 6.6 Hz, 3H), 0.858 (d, 6.6 Hz, 3H), δ_C 39.46

(replacing 39.34), 27.98 (replacing 27.50), 22.77, 22.53.

Similar treatment of **6c** (18 mg) gave **7c** (14 mg, 86% yield, 99% purity): m.p., 130–131°C; single component by TLC (R_f 0.35, SS-4); high-resolution MS, calcd. for $C_{27}H_{40}D_6O_2$, 408.3874, found 408.3874; MS, Table 5; IR, 3420, 2936, 2866, 2216, 1468, 1447, 1375, 1057 cm^{-1}; NMR, δ_H (50 mM solution) 3.695 (dddd, 11.8, 10.3, 4.8, 4.4 Hz), 3.057 (br dd, 2.9, 1.0 Hz), 2.075 (dddd, 14.3, 3.7, 2.7, 0.7 Hz), 2.038 (br dd, 13.0, 11.8 Hz), 1.479 (qd, 11.0, 4.0 Hz), 1.426 (ddd, 13.1, 4.8, 2.3 Hz), 1.196 (ddd, 14.6, 11.0, 1.2 Hz), 0.993 (s, 3H), 0.889 (d, 6.6 Hz, 3H), 0.639 (d, 0.4 Hz, 3H); δ_C 69.42, 63.71, 62.92, 56.22, 56.20, 51.32, 42.27, 42.22, 39.82, 39.34, 37.21, 36.12, 35.71, 34.84, 32.60, 31.04, 29.76, 28.13, 27.50, 24.17, 23.79, 21.98, 18.66, 17.03, 11.74 (no signals observed for C-26, C-27).

*2.8. 25,26,26,26,27,27,27-Heptafluoro-5α-cholestane-3β,5,6β-triol (**8b**), 5α-cholestane-3β,5,6β-triol (**8a**), and [26,26,26,27,27,27-$^2H_6$]5α-cholestane-3β,5,6β-triol (**8c**)*

A suspension of **1b** (40 mg, 0.078 mmol) in 95% formic acid (2 ml) was heated with stirring to 75°C for 5 min; an oil layer formed on the surface. The mixture was cooled to 25°C and 30% hydrogen peroxide (0.5 ml) was added dropwise. The reaction was stirred for 6 h, followed by addition of boiling water (50 ml). The white precipitate that formed was collected by filtration, dried in vacuo, and dissolved in methanol (5 ml). Sodium hydroxide (10 mg) was added, and the resulting solution was stirred at room temperature for 0.5 h. TLC analysis (SS-7) showed a predominant product at R_f 0.21. The methanol was evaporated, and the resulting residue was dissolved in dichloromethane (10 ml), followed by washing with brine (10 ml) and drying over anhydrous sodium sulfate. Evaporation gave a white solid that was purified by MPLC (1000 × 10 mm i.d. column; elution with methanol–chloroform 5.95). Evaporation of fractions 24–29 gave **8b** as a white solid (32 mg, 75% yield, ~98% purity): m.p., 260–261°C; single component by TLC (R_f 0.09, SS-6 and R_f 0.21, SS-7); high-resolution MS, calcd. for $C_{27}H_{41}O_3F_7$, 546.2944, found 546.2940; MS, 546† (2, M^+), 528*† (49), 510*† (100), 495*† (20), 492*† (27), 474† (10, $M-C_4H_8O$), 457† (10, $M-C_4H_9O$), 429† (6, $M-C_6H_{13}O$), 401† (5, $C_{20}H_{28}F_7$), 373† (12, $C_{18}H_{24}F_7$), 319† (5, $C_{14}H_{18}F_7$), 271*† (6), 253*† (4), 247† (13, $C_{16}H_{23}O_2$), 244† (25, $C_{17}H_{24}O$), 239† (11, SC), 229† (18, $C_{16}H_{21}O$), 211† (13, $C_{16}H_{19}$), 107† (32, C_8H_{11}); IR, 3434, 3399, 2946, 2916, 2870, 1468, 1445, 1379, 1316, 1221, 1159, 1046 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4; ^{19}F NMR, δ_F −184.15 (dd of septet, 21.2, 19.9, 6.6 Hz), −76.74 and −76.94 (A_3B_3 portion of A_3B_3X system (Swaminathan et al., 1993)).

Similar treatment of **1a** (1.0 g) gave **8a** as a white solid (0.75 g, 69% yield, >99% purity): m.p., 233.5–235°C (lit. 236–238°C (Baxter and Spring, 1943)); single component by TLC (R_f 0.10, SS-6); MS, 420 (4, M^+), 402* (100), 388 (10), 384* (88), 369* (30), 366* (11), 348 (10), 331 (17), 303 (9), 271* (13), 262 (25), 253* (5), 247 (34), 244 (28), 229 (29), 211 (17), 193 (4), 107 (39); ^1H and ^{13}C NMR data identical (±0.003 ppm for ^1H, ±0.04 ppm for ^{13}C) to those of **8c** except for the occurrence of signals at δ_H 1.515 (nonet, 6.6 Hz), 0.865 (d, 6.6 Hz, 3H), 0.861 (d, 6.6 Hz, 3H), δ_C 39.47 (replacing 39.34), 27.99 (replacing 27.50), 22.81, 22.55.

Similar treatment of **1c** (20 mg) gave **8c** as a white solid (17 mg, 78% yield, 99% purity): m.p., 235–236°C; single component by TLC (R_f 0.10, SS-6); high-resolution MS, calcd. for $C_{27}H_{42}D_6O_3$, 426.3980, found 426.3980; MS, 426† (3, M^+), 408*† (100), 390*† (83), 375*† (24), 372*† (16), 354† (10, $M-C_4H_8O$), 337† (20, $M-C_4H_9O$), 309† (7, $M-C_6H_{13}O_2$), 289*† (9), 281† (4, $C_{20}H_{29}D_6$), 271*† (13), 268† (17, $M-C_8H_{14}O_3$), 253† (14, $C_{18}H_{25}D_6$ and $C_{19}H_{25}$), 247† (31, $C_{16}H_{23}O_2$), 244† (26, $C_{17}H_{24}O$), 229† (26, $C_{16}H_{21}O$), 211† (18, $C_{16}H_{19}$), 199† (9, $C_{14}H_{19}D_6$), 107† (36, C_8H_{11}); IR, 3583, 3562, 3543, 3420, 3400, 3273, 2937, 2866, 2214, 1647, 1456, 1375, 1292, 1085, 1043, 1014 cm^{-1}; NMR, δ_H 4.098 (tt, 11.0, 5.4 Hz), 3.542 (t, 2.9 Hz), 2.086 (dd, 12.9, 11.1 Hz), 1.999 (dddd, 12.6, 3.8, 3.1, 0.4 Hz), ~1.632 (ddd, 14.1, 11.4, 3.4 Hz), 1.616 (ddd, 12.8, 5.2, 1.9 Hz), ~1.594 (ddd, 14.2, 5.1, 2.7 Hz), 1.184 (d, 0.4 Hz, 3H), 0.904 (d, 6.6 Hz, 3H),

0.679 (d, 0.5 Hz, 3H), δ_D 0.84 (s), δ_C 76.08, 76.05, 67.62, 56.20, 55.90, 45.87, 42.72, 40.70, 39.91, 39.34, 38.27, 36.13, 35.78, 34.51, 32.35, 30.83, 30.17, 28.20, 27.50, 24.12, 23.83, 21.15, 18.64, 16.89, 12.14 (no signals observed for C-26, C-27).

*2.9. [16α-^{2}H]-(25R)-Bis(3β,26-tert-butyldimethylsilyloxy)cholest-5-en-16β-ol (**10**) and [16β-^{2}H]-(25R)-bis(3β,26-tert-butyldimethylsilyloxy)cholest-5-en-16α-ol (**11**)*

To a solution of 16-ketosterol **9** (2.50 g, 3.9 mmol) in anhydrous diethyl ether (200 ml) at 0°C was added lithium aluminum deuteride (0.16 g, 3.8 mmol) under nitrogen. The reaction mixture was stirred at room temperature overnight, followed by addition of five drops of water. The resulting slurry was filtered and washed with diethyl ether (50 ml). The filtrate was evaporated to a residue that was subjected to MPLC (500 mm × 25 mm i.d. column; elution with ethyl acetate–hexane 4:96). Fractions 30–44 gave **10** as a white solid (1.80 g, 72% yield, 99% purity, > 99% d_1 by ^{1}H NMR): m.p., 121.5–122.5°C (lit. m.p. for protio analog, 123–124°C (Kim et al., 1989)); single component by TLC (R_f 0.57, SS-1); high-resolution MS, calcd. for $C_{39}H_{73}DO_3Si_2$, 647.5239, found 647.5228; MS (> 99% d_1), 647† (1, M^+), 632*† (3), 629*† (2), 590*† (68), 572*† (20), 500*† (5), 498*† (3, $M - H_2O$–TBDMSO), 458*† (45), 440*† (4), 366*† (6, 498 − TBDMSOH), 330 (8), 256† (9, $C_{19}H_{26}D$), 254† (12, $C_{19}H_{24}D$), 213† (8, $C_{16}H_{19}D$), 159† (20, $C_{12}H_{15}$), 115† (8, $C_6H_{15}Si$), 105† (20, C_8H_7D), 75† (100, C_2H_7OSi); IR, 3628, 2957, 2930, 2883, 2856, 1469, 1384, 1251, 1076, 837, 775 cm^{-1}; ^{1}H and ^{13}C NMR identical (± 0.002 ppm for ^{1}H, ± 0.02 ppm for ^{13}C) to those of the undeuterated sterol[2] (Kim et al., 1989) except for small upfield shifts for ring D protons, H-20, C-15 and C-17 (0.11–0.13 ppm) and C-16 (0.5 ppm). These ^{13}C signals appeared as a weak multiplets, and the residual H-16α signal at δ_H 4.35 integrated for 0.004 H.

[2] NMR data were compared with recent spectra of undeuterated 25- and 26-hydroxysterols in dilute solution; these unpublished data differ slightly from those in the cited references.

Fractions 50–60 gave **11** as a white solid (0.50 g, 20% yield, ≥ 99% purity): m.p.: 100–101°C; single component by TLC (R_f 0.47, SS-1); high-resolution MS, calcd. for $C_{39}H_{73}DO_3Si_2$, 647.5239, found 647.5240; MS (> 99% d_1), 647† (1, M^+), 632*† (4), 629*† (4), 590*† (73), 572*† (40), 500*† (10), 498*† (11, $M - H_2O$–TBDMSO), 458*† (65), 440*† (6), 366*† (10, 498 − TBDMSOH), 330 (10), 256† (16, $C_{19}H_{26}D$), 254† (18, $C_{19}H_{24}D$), 213† (13, $C_{16}H_{19}D$), 159† (30, $C_{12}H_{15}$), 115† (12, $C_6H_{15}Si$), 105† (27, C_8H_7D), 75† (100, C_2H_7OSi); IR, 3622, 2949, 2931, 2885, 1471, 1383, 1249, 1085, 839, 773 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 1 and 2) showed absence of signals for C-16 and H-16β (< 0.01H).

*2.10. [16α-^{2}H]-(25R)-Bis(3β,26-tert-butyldimethylsilyloxy)cholest-5-en-16β-ol methanesulfonate ester (**12**)*

To a solution of **10** (1.71 g, 2.64 mmol) in pyridine (50 ml) at 0°C was added methanesulfonyl chloride (1.4 ml, 18.1 mmol) dropwise under nitrogen. The reaction mixture was stirred at room temperature for 24 h, poured into ice-water (200 ml), and extracted with dichloromethane (3 × 100 ml). The combined extracts were washed with cold 5% sulfuric acid (3 × 100 ml) and brine (30 ml), dried over anhydrous sodium sulfate, and evaporated to give **12** as a white solid (2.0 g, 104% yield): TLC (R_f 0.45, SS-1).

*2.11. [16,16-$^2H_2$]-(25R)-Bis(3β,26-tert-butyldimethylsilyloxy)cholest-5-ene (**13**) and [16-^{2}H]-(25R)-bis(3β,26-tert-butyldimethylsilyloxy)cholesta-5,16-diene (**14**)*

A solution of crude **12** (1.9 g, 2.6 mmol) in diethyl ether (20 ml) was added dropwise to a solution of lithium aluminum deuteride (94 mg, 2.2 mmol) in diethyl ether (100 ml) at 0°C. The reaction mixture was stirred at room temperature for 5 h and quenched with water (five drops). The inorganic precipitate was removed by filtration and thoroughly washed with diethyl ether. Evaporation gave a white powder (1.6 g): single component by TLC (R_f 0.85, ethyl acetate–hexane 5:95); 9:2 mixture of **13** and **14** by ^{1}H NMR. Owing to

the hydrophobicity of the TBDMS groups, the mixture was not retained on alumina–$AgNO_3$ (elution with hexane), and the sample was converted to the diacetate derivative for purification as described below.

2.12. [16,16-$^2H_2$]-(25R)-Cholest-5-ene-3β,26-diol diacetate (**2e**) and [16-^{2}H]-(25R)-cholesta-5,16-diene-3β,26-diol diacetate (**15**)

Tetrabutylammonium fluoride (15 ml, 1.0 M solution in tetrahydrofuran) was added to the 9:2 mixture of **13** and **14** in tetrahydrofuran (100 ml). The reaction mixture was stirred for 24 h and analyzed by TLC, which showed disappearance of **13** and **14** and the formation of polar material with the same mobility as **1d** (R_f 0.62, SS-5). Extraction with ethyl acetate (2×150 ml) followed by washing with water (100 ml) and brine (50 ml), drying over anhydrous sodium sulfate, and evaporation to dryness furnished a white solid (1.0 g): single component by TLC (R_f 0.6, SS-5). To a solution of the crude diol in pyridine (50 ml) was added acetic anhydride (5 ml). The reaction mixture was stirred overnight at room temperature, poured into ice-water (200 ml), and extracted with dichloromethane (3×100 ml). The combined organic extracts were washed with cold 5% sulfuric acid (3×100 ml) and brine (30 ml) and dried over anhydrous sodium sulfate. Evaporation gave a white solid that was subjected to MPLC (500×25 mm i.d. column, elution with ethyl acetate–hexane 4:96). Evaporation of fractions 42–60 gave **2e** as a white solid (0.90 g, 70% yield from **12**, $\geq$99% purity): m.p., 126–127°C; single component by TLC (R_f 0.62, SS-2); high-resolution MS, calcd. for $C_{29}H_{44}D_2O_2$, 428.3623 (M − 60), found 428.3625; MS ($\sim$99% d_2), 428*† (100), 413*† (10), 368*† (25), 307† (14, M − $C_{11}H_{17}O_2$), 257*† (13), 255*† (8), 213† (15, $C_{16}H_{21}$), 158† (35, $C_{12}H_{14}$), 145† (33, $C_{11}H_{13}$), 105† (24, C_8H_9); IR, 2945, 2910, 2872, 2850, 2831, 1736, 1466, 1377, 1365, 1238, 1033 cm^{-1}; ^{1}H and ^{13}C NMR data essentially identical to those of the undeuterated sterol[2] (Siddiqui et al., 1992) except for the deuterium isotope effects and the absence of signals and couplings for C-16, H-16α, and H-16β; ^{2}H NMR ($\pm$0.003 ppm), 1.819 (s), 1.237 (s).

Fractions 80–86 gave **15** as a white solid (0.20 g, 16% yield from **12**, $\sim$98% purity): m.p., 126–127°C; single component by TLC (R_f 0.62, SS-2); high-resolution MS, calcd. for $C_{30}H_{44}DO_4$, 470.3381 (M − 15), found 470.3392; MS ($\sim$99% d_1), 470*† (2), 425*† (72), 410*† (46), 365*† (2), 358† (3, $C_{25}H_{26}O_2$), 350*† (4), 314*† (4), 304† (4, M − $C_{11}H_{17}O_2$), 282† (10, $C_{21}H_{28}D$), 266† (3, $C_{20}H_{24}D$), 254*† (72), 236† (21, $C_{16}H_{26}DO$ and $C_{18}H_{18}D$), 221 (37), 212† (10, $C_{16}H_{18}D$), 180† (79, $C_{14}H_{10}D$), 165† (86, $C_{12}H_{19}D$), 57† (100, C_4H_9); IR, 2960, 2939, 2924, 2910, 2852, 1732, 1465, 1448, 1438, 1369, 1246, 1035 cm^{-1}; ^{1}H NMR (50 mM in $CDCl_3$), δ_H 5.397 (br dq, 5.2, 1.3 Hz), 5.29 (m, 0.01H), 4.608 (m), 3.935 (dd, 10.7, 5.9 Hz), 3.837 (dd, 10.7, 6.9 Hz), 2.34 (m), 2.32 (m), 2.050 (s, 3H), 2.034 (s, 3H), 1.058 (d, 0.5 Hz, 3H), 0.991 (d, 6.9 Hz, 3H), 0.911 (d, 6.8 Hz, 3H), 0.781 (d, 0.4 Hz, 3H); ^{13}C NMR, Table 2.

2.13. [16,16-$^2H_2$]-(25R)-Cholest-5-ene-3β,26-diol (**1e**)

To a solution of **2e** (300 mg, 0.61 mmol) in methanol (50 ml) was added sodium carbonate (100 mg, 1.0 mmol). The reaction mixture was stirred at room temperature overnight, concentrated in vacuo, and extracted with ethyl acetate (100 ml). The organic layer was washed with water (2×50 ml) and dried over sodium sulfate. Evaporation gave a white solid that was subjected to MPLC (500×10 mm i.d. column; elution with ethyl acetate–hexane 2:8). Evaporation of fractions 34–51 gave **1e** as a white solid (220 mg, 89% yield, $\geq$99% purity): m.p., 173–174°C; single component by TLC (R_f 0.60, SS-5); high-resolution MS, calcd. for $C_{27}H_{44}D_2O_2$, 404.3623, found 404.3626; MS (96% d_2, 2% d_1, 2% d_0),[3] 404 (100, M^+), 389*† (30), 386*† (55), 371*† (28), 347† (4, M − C_3H_5O), 344† (7, M − C_3H_8O), 319† (35, M − C_5H_9O), 293† (66, M − $C_7H_{11}O$), 275*† (22),

[3] Mass spectra of diacetates **2e** and **16e** showed 100% d_2, but lower values (90–98% d_2)were sometimes observed for diols **1e** and **17e**. Because acetate hydrolysis should not affect deuterium at C-16, the lower values were attributed to dehydrogenation (M − 2), which was observed in low and variable abundance for the undeuterated diols **1d** and **17d**.

272† (9, $C_{19}H_{24}D_2O$), 265† (13, $C_{18}H_{29}D_2O$), 257*† (26), 231† (18, $C_{16}H_{23}O$), 228† (10, $C_{17}H_{24}$), 213† (34, $C_{16}H_{21}$), 199† (9, $C_{15}H_{15}D_2$), 159† (32, $C_{12}H_{15}$), 145† (45, $C_{11}H_{13}$), 119† (37, C_9H_{11}); IR, 3329, 3319, 2960, 2933, 2897, 2877, 2850, 1464, 1437, 1375, 1357, 1057, 1022 cm^{-1}; ^1H NMR data essentially identical to those of the undeuterated sterol[2] (Kim et al., 1989) except for deuterium isotope effects and the absence of signals and couplings for H-16α, and H-16β; ^2H NMR (± 0.003 ppm), 1.815 (s), 1.244 (s).

*2.14. 3β-Acetoxy-25,26,26,26,27,27,27-heptafluorocholest-5-en-7-one (**16b**), 3β-acetoxycholest-5-en-7-one (**16a**), [26,26,26,27,27,27-$^2H_6$]3β-acetoxycholest-5-en-7-one (**16c**), (25R)-3β,26-diacetoxycholest-5-en-7-one (**16d**), and [16,16-$^2H_2$]-(25R)-3β,26-diacetoxycholest-5-en-7-one (**16e**)*

To a solution of **2b** (170 mg, 0.31 mmol) in benzene (100 ml; dried over sodium) were added pyridinium chlorochromate (1.6 g, 7.4 mmol) and molecular sieve (0.1 g; type 3A) in one portion. The reaction mixture was refluxed under nitrogen for 24 h, filtered through a pad of silica gel (230–400 mesh) with thorough elution using ethyl acetate–hexane 1:1. The filtrate was dried over anhydrous sodium sulfate and evaporated to a white solid that was subjected to MPLC (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 3:97 (1000 ml) and ethyl acetate–hexane 6:94). Evaporation of fractions 52–60 gave **16b** as a white solid (113 mg, 65% yield, ≥99% purity): single component by TLC (R_f 0.27, SS-1); high-resolution MS, calcd. for $C_{27}H_{35}OF_7$ (M − 60), 508.2576, found 508.2561; MS, 568† (0.1, M^+), 508*† (100), 493*† (6), 489*† (4), 466† (2, M − $C_5H_{10}O_2$), 297† (3, M − CH_3COOH–$C_6H_6F_7$), 269*† (10), 242† (3, $C_{17}H_{22}O$), 239† (2, SC), 229† (4, $C_{16}H_{21}O$), 227† (5, $C_{16}H_{19}O$), 174† (42, $C_{12}H_{14}O$), 161† (30, $C_{11}H_{13}O$); IR, 2945, 2874, 2862, 1734, 1670, 1464, 1377, 1238, 1157, 1039 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4.

Similar treatment of **2a** (450 mg) gave **16a** as a white solid (300 mg, 65% yield, ≥99% purity): m.p., 161–162°C (lit. 161–163°C (Dauben et al., 1969), 162–163°C (Kudo et al., 1989), 163–164°C (Morand and Van Tongerloo, 1973)); single component by TLC (R_f 0.21, SS-1); MS, 442 (0.6, M^+), 382* (100), 367* (11), 340 (3), 297* (3), 269* (20), 242 (5), 229 (9), 227 (9), 174 (73), 161 (33); ^1H NMR data identical (± 0.001 ppm) to those of **16c** except for the occurrence of signals at δ_H 1.517 (nonet, 6.6 Hz), 0.866 (d, 6.6 Hz, 3H), 0.862 (d, 6.6 Hz, 3H).

Similar treatment of **2c** (170 mg) gave **16c** as a white solid (110 mg, 63% yield; 98% purity): m.p., 156–157°C; single component by TLC (R_f 0.21, SS-1); high-resolution MS, calcd. for $C_{29}H_{40}D_6O_3$, 448.3824, found 448.3836; MS, 448† (1, M^+), 388*† (100), 373*† (5), 346† (2, M − $C_5H_{10}O_2$), 297 (2), 269*† (14), 253 (1, $C_{18}H_{25}D_6$), 242† (3, $C_{17}H_{22}O$), 229† (6, $C_{16}H_{21}O$), 227† (6, $C_{16}H_{19}O$), 174† (59, $C_{12}H_{14}O$), 161† (26, $C_{11}H_{13}O$); IR, 2939, 2906, 2885, 2868, 2214, 1734, 1703, 1467, 1442, 1381, 1255, 1037 cm^{-1}; NMR, δ_H (50 mM solution) 5.702 (d, 1.7 Hz), 4.715 (tdd, 11.5, 5.1, 4.3 Hz), 2.547 (ddd, 14.1, 5.1, 2.3 Hz), 2.467 (ddd, 13.9, 11.6, 2.0 Hz), 2.40 (m), 2.231 (dd, 12.0, 10.8 Hz), 2.051 (s, 3H), 1.683 (distorted dddd, 14.2, 12.5, 11.8, 3.6), 1.209 (d, 0.6 Hz, 3H), 0.922 (d, 6.6 Hz, 3H), 0.682 (s, 3H), δ_C 201.94, 170.26, 163.82, 126.69, 72.19, 54.75, 49.93, 49.79, 45.40, 43.09, 39.32, 38.64, 38.29, 37.73, 36.16, 35.98, 35.70, 28.52, 27.48, 27.33, 26.28, 23.80, 21.24, 21.15, 18.84, 17.24, 11.95 (no signals observed for C-26, C-27).

Similar treatment of **2d** (300 mg) gave **16d** as a white solid (212 mg, 69% yield; >99% purity): m.p., 119.5–120.5°C (lit. 121–122°C (Noll et al., 1973); single component by TLC (R_f 0.21, SS-2); high-resolution MS, calcd. for $C_{31}H_{48}O_5$, 500.3502, found 500.3523; MS, 500† (1, M^+), 440*† (100), 425*† (3), 380*† (3), 365*† (1), 269*† (15), 242† (3, $C_{17}H_{22}O$), 229† (6, $C_{16}H_{21}O$), 228† (5, $C_{16}H_{20}O$), 227† (6, $C_{16}H_{19}O$), 213† (3, $C_{15}H_{17}O$), 187† (16, $C_{13}H_{15}O$), 174† (45, $C_{12}H_{14}O$), 161† (23, $C_{11}H_{13}O$); IR, 2949, 2874, 1736, 1676, 1643, 1462, 1371, 1238, 1030 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2.

Similar treatment of **2e** (500 mg) gave, after MPLC (1000 × 10 mm i.d. column, elution with ethyl acetate–hexane 6:94) and evaporation of fractions 67–79, **16e** as a white solid (360 mg, 70% yield, 99% purity): mp, 117–118.5°C; single

component by TLC (*R*f 0.21, SS-2); high-resolution MS, calcd. for $C_{31}H_{46}D_2O_5$, 502.3627, found 502.3626; MS ($\sim$99% d_2), 502† (2, M^+), 442*† (100), 427*† (3), 382*† (3), 367*† (2), 271*† (17), 242† (6, $C_{17}H_{22}O$), 229† (6, $C_{16}H_{21}O$), 228† (8, $C_{16}H_{20}O$), 227† (8, $C_{16}H_{19}O$), 213† (3, $C_{15}H_{13}D_2O$), 174† (62, $C_{12}H_{14}O$), 161† (31, $C_{11}H_{13}O$); IR, 2951, 2881, 1736, 1678, 1464, 1377, 1238, 1031 cm^{-1}; ^{1}H and ^{13}C NMR data essentially identical to those of **16d** except for deuterium isotope effects and the absence of signals and couplings for C-16, H-16α, and H-16β.

*2.15. 3β-Hydroxy-25,26,26,26,27,27,27-heptafluorocholest-5-en-7-one (**17b**), 3β-hydroxycholest-5-en-7-one (**17a**), [26,26,26,27,27,27-$^2H_6$]3β-hydroxycholest-5-en-7-one (**17c**), (25R)-3β,26-dihydroxycholest-5-en-7-one (**17d**), and [16,16-$^2H_2$]-(25R)-3β,26-dihydroxycholest-5-en-7-one (**17e**)*

As described above for **4b**, saponification of **16b** (50 mg, 0.088 mmol) in methanol (10 ml) and sodium carbonate (20 mg) gave after workup **17b** as a white solid (41 mg, 89% yield, 98% purity): m.p., 164–165°C; single component by TLC (R_f 0.48, SS-6); high-resolution MS, calcd. for $C_{27}H_{37}O_2F_7$, 526.2682, found 526.2674; 526† (100, M^+), 511* (3), 508*† (25), 493*† (21), 467† (2, $M-C_3H_7O$), 315† (2, $M-C_6H_6F_7$), 287*† (5), 269*† (4), 245† (3, $C_{16}H_{21}O_2$), 239† (6, SC), 205† (4, $C_{13}H_{17}O_2$), 174† (7, $C_{12}H_{14}O$), 161† (13, $C_{11}H_{13}O$); IR, 3522, 2939, 2864, 1664, 1464, 1311, 1219, 1159.3, 1058, 943, 719 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 3 and 4.

Similar treatment of **16a** (100 mg) gave **17a** as a white solid (85 mg, 94% yield, $\geq$99% purity): m.p., 171–172°C (lit. 171–172°C (Parish and Scott, 1983)); single component by TLC (R_f 0.49, SS-6); MS, 400 (100, M^+), 385* (5), 382* (10), 367* (14), 341 (2), 287* (12), 269* (4), 245 (7), 174 (12), 161 (26); ^{1}H NMR data identical ($\pm$0.004 ppm) to those of **17c** except for the occurrence of signals at δ_H 1.517 (nonet, 6.6 Hz), 0.866 (d, 6.6 Hz, 3H), 0.861 (d, 6.6 Hz, 3H).

Similar treatment of **16c** (20 mg) gave **17c** as a white solid (16 mg, 88% yield, 99% purity): m.p., 165.5–166.5°C; single component by TLC (R_f 0.49, SS-6); high-resolution MS, calcd. for $C_{27}H_{38}D_6O_2$, 406.3718, found 406.3736; MS, 406† (100, M^+), 391*† (3), 388*† (45), 373*† (17), 347† (2, $M-C_3H_7O$), 287*† (10), 269*† (7), 245† (7, $C_{16}H_{21}O_2$), 205† (8, $C_{13}H_{17}O_2$), 174† (44, $C_{12}H_{14}O$), 161† (39, $C_{11}H_{13}O$); IR, 3435, 2936, 2866, 2214, 1665, 1462, 1439, 1379, 1230, 1057 cm^{-1}; NMR, δ_H (50 mM solution) 3.674 (tdd, 11.2, 4.8, 4.1 Hz), 2.507 (ddd, 13.9, 4.7, 2.3 Hz), 2.400 (ddd, 13.8, 11.4, 2.0 Hz), 2.40 (m), 2.239 (dd, 12.4, 10.7 Hz), 2.034 (distorted dt, 12.7, 3.5 Hz), 1.198 (d, 0.6 Hz, 3H), 0.921 (d, 6.7 Hz, 3H), 0.683 (d, 0.4 Hz, 3H), δ_C (25°C) 202.35, 165.15, 126.07, 70.48, 54.77, 49.94, 49.90, 45.39, 43.08, 41.79, 39.32, 38.68, 38.26, 36.32, 36.16, 35.69, 31.15, 28.52, 27.48, 26.30, 23.80, 21.20, 18.85, 17.29, 11.95 (no signals observed for C-26, C-27).

Similar treatment of **16d** (60 mg) gave **17d** as a white solid (45 mg, 90% yield): m.p., 173–174°C; single component by TLC (R_f 0.30, SS-5); high-resolution MS, calcd. for $C_{27}H_{44}O_3$, 416.3290, found 416.3284; MS, 416† (100, M^+), 401*† (3), 398*† (20), 383*† (11), 380*† (5), 365*† (2), 359 (3), 358† (3, $C_{24}H_{38}O_3$), 287*† (17), 269*† (9), 260† (4, $C_{17}H_{24}O_2$), 247† (7, $C_{16}H_{23}O_2$), 245† (8, $C_{16}H_{21}O_2$), 227† (6, $C_{16}H_{19}O$), 213† (5, $C_{15}H_{17}O$), 209† (6, $C_{14}H_{25}O$ and $C_{16}H_{17}$), 205† (16, $C_{13}H_{17}O_2$), 192† (35, $C_{12}H_{16}O_2$), 187† (24, $C_{13}H_{15}O$); IR, 3365, 2935, 2866, 1670, 1460, 1373, 1356, 1181, 1076, 1045, 1024 cm^{-1}; ^{1}H NMR, δ_H 5.693 (d, 1.7 Hz), 3.678 (tq, 11.3, 4.4 Hz), 3.504 (dt, 10.6, 5.2 Hz), 3.422 (ddd, 10.7, 6.5, 4.5 Hz), 1.199 (d, 0.6 Hz), 0.925 (d, 6.6 Hz), 0.914 (d, 6.7 Hz), 0.683 (d, 0.4 Hz).

Similar treatment of **16e** (50 mg) gave, after MPLC (300 × 10 mm i.d. column, elution with ethyl acetate–hexane 25:75) and evaporation of fractions 25–39, **17e** as a white solid (38 mg, 91% yield, $\sim$99% purity): m.p., 172–173.5°C; single component by TLC (R_f 0.30, SS-5); high-resolution MS, calcd. for $C_{27}H_{42}D_2O_3$, 418.3416, found 418.3416; MS ($\sim$99% d_2),[3] 418† (100, M^+), 400*† (8), 385*† (5), 289*† (15), 271*† (4), 260† (6, $C_{17}H_{24}O_2$), 247† (6, $C_{16}H_{23}O_2$), 245† (9, $C_{16}H_{21}O_2$), 227† (4, $C_{16}H_{19}O$), 213† (4, $C_{15}H_{13}D_2O$), 205† (11, $C_{13}H_{17}O_2$), 192† (33,

$C_{12}H_{12}D_2O_2$), 187† (14, $C_{13}H_{15}O$), 135 (20); IR, 3365, 2964, 2933, 2877, 2864, 1668, 1635, 1465, 1359, 1292, 1184, 1076, 1058, 1026 cm^{-1}; ^1H NMR data essentially identical to those of **17d** except for deuterium isotope effects and the absence of signals and couplings for H-16α, and H-16β.

*2.16. 25,26,26,26,27,27,27-Heptafluorocholest-5-ene-3β,7α-diol diacetate (**18b**), cholest-5-ene-3β,7α-diol diacetate (**18a**), and [26,26,26,27,27,27-$^2H_6$]cholest-5-ene-3β,7α-diol diacetate (**18c**) and their 7β epimers **19b**, **19a**, and **19c***

To a solution of **16b** (100 mg, 0.19 mmol) in anhydrous diethyl ether (30 ml) was added lithium aluminum hydride (29 mg, 0.76 mmol) in one portion. The reaction mixture was stirred under nitrogen at room temperature overnight. Water (two drops) was added to decompose the excess lithium aluminum hydride, and the inorganic precipitate was removed by filtration. The filtrate was evaporated to a white solid that was dissolved in pyridine (2 ml) and acetic anhydride (0.3 ml). The reaction mixture was heated to 100°C for 1 h, cooled to room temperature, and evaporated to a residue that was subjected to MPLC on alumina–$AgNO_3$ (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 5:95 (1000 ml) and ethyl acetate–hexane 7:93). Evaporation of fractions 33–45 furnished **18b** as a white solid (20 mg, 19% yield, ≥98% purity): m.p., 98–100°C; single component by TLC (R_f 0.61, SS-2); high-resolution MS, calcd. for $C_{29}H_{39}O_2F_7$ (M − 60), 552.2838, found 552.2838; MS, Table 6; IR, 2957, 2895, 2854, 1726, 1442, 1238, 1157, 1031, 1014, 935 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4.

Fractions 90–113 gave **19b** as a white solid (65 mg, 60% yield, ≥98% purity): m.p., 59–62°C; single component by TLC (R_f 0.63, SS-2); high-resolution MS, calcd. for $C_{29}H_{39}O_2F_7$, 552.2838 (M − 60), found 552.2834, MS, Table 6; IR, 2947, 2872, 1736, 1467, 1440, 1373, 1311, 1240, 1159, 1022, 949 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4.

Similar treatment of **16a** (270 mg) gave **18a** (fractions 12–20) as a white solid (63 mg, 21% yield, ≥98% purity): m.p., 121–122°C (lit. 122–123°C (Nickon and Mendelson, 1965)); single component by TLC (R_f 0.66, SS-2); MS, Table 6; ^1H and ^{13}C NMR data identical (±0.001 ppm for ^1H, ±0.01 ppm for ^{13}C) to those of **18c** except for the occurrence of signals at δ_H 1.518 (nonet, 6.6 Hz), 0.869 (d, 6.6 Hz, 3H), 0.864 (d, 6.6 Hz, 3H), δ_C 39.45 (replacing 39.31), 27.99 (replacing 27.50), 22.81, 22.53.

Fractions 46–72 gave **19a** as a white solid (180 mg, 61% yield, ≥98% purity): m.p., 106–108°C (lit. 106°C (Nickon and Mendelson, 1965)); single component by TLC (R_f 0.68, SS-2); MS, Table 6; ^1H and ^{13}C NMR data identical (± 0.002 ppm for ^1H, ±0.01 ppm for ^{13}C) to those of **19c** except for the occurrence of signals at δ_H 1.515 (nonet, 6.6 Hz), 0.865 (d, 6.6 Hz, 3H), 0.860 (d, 6.6 Hz, 3H), δ_C 39.43 (replacing 39.30), 27.96 (replacing 27.47), 22.79, 22.53.

Similar treatment of **16c** (85 mg) gave **18c** (fractions 30–40) as a colorless oil (20 mg, 21% yield, ≥98% purity): single component by TLC (R_f 0.66, SS-2); high-resolution MS, calcd. for $C_{29}H_{40}D_6O_2$ (M − 60), 432.3874, found 432.3879; MS, Table 6; IR, 2941, 2895, 2870, 2721, 2677, 2214, 1730, 1442, 1369, 1238, 1221, 1031, 1014, cm^{-1}; NMR, δ_H (50 mM solution) 5.585 (dt, 5.2, 1.3 Hz), 4.962 (br t, ~4.6 Hz), 4.674 (m), 2.36 (m, 2H), 2.040 (s, 3H), 2.036 (s, 3H), 2.008 (br dt, 12.6, 4.0, 3.0 Hz), 1.013 (s, 3H), 0.925 (d, 6.6 Hz, 3H), 0.669 (s, 3H), δ_C (25°C) 170.83, 170.45, 146.46, 120.82, 73.13, 68.27, 55.90, 49.13, 43.00, 42.22, 39.31, 38.98, 37.79, 37.23, 36.46, 36.14, 35.75, 35.70, 28.12, 27.50, 27.46, 24.03, 23.85, 21.36, 21.36, 20.63, 18.70, 18.14, 11.43 (no signals observed for C-26, C-27).

Fractions 70–85 gave **19c** as a colorless oil (65 mg, 70% yield, ≥98% purity): single component by TLC (R_f 0.66, SS-2); high-resolution MS, calcd. for $C_{29}H_{40}D_6O_2$ (M − 60), 432.3874, found 432.3864; MS, Table 6; IR, 2945, 2872, 2854, 2214, 1736, 1456, 1373, 1240, 1030, 1020, 949 cm^{-1}; NMR, δ_H (50 mM solution) 5.240 (m), 5.034 (br dt, 8.6, 2.0 Hz), 4.601 (distorted dddd, 11.5, 10.6, 6.3, 4.3 Hz), 2.339 (distorted ddd, 13.2, 6.0, 2.0 Hz), 2.319 (distorted ddt, 13.2, 10.5, 2.0 Hz), 2.028 (s, 3H), 2.019 (s, 3H), 1.692 (ddd, 11.9, 10.8, 8.7 Hz), 1.085 (d, 0.4 Hz, 3H),

Table 6
Comparison of mass spectral data for 7α and 7β hydroxycholesterol (**20a** and **21a**), their 25,26,26,26,27,27,27-heptafluoro (F_7) derivatives **20b** and **21b**, their 26,26,26,27,27,27-hexadeuterio (d_6) derivatives **20c** and **21c**, and diacetate derivatives of the six diols[a]

	3β,7-Diols ($R = H$)						3β,7-Diacetates ($R = CH_3CO$)					
	F_7-7α **20b**	F_7-7β **21b**	7α **20a**	7β **21a**	d_6-7α **20c**	d_6-7β **21c**	F_7-7α **18b**	F_7-7β **19b**	7α **18a**	7β **19a**	d_6-7α **18c**	d_6-7β **19c**
M^+	528 (3)	(2)	402 (1)	(0.3)	408 (3)	(5)	612 (−)	(−)	486 (−)	(−)	492 (−)	(−)
M−ROH	510 (100)	(40)	384 (29)	(9)	390 (67)	(75)	552 (13)	(11)	426 (6)	(10)	432 (1)	(9)
M−ROH–Me	495 (3)	(4)	369 (4)	(2)	375 (3)	(7)	537 (−)	(−)	411 (0.2)	(0.4)	417 (0.5)	(0.3)
M−AcOH–C_2H_2O							510 (79)	(74)	384 (61)	(36)	390 (67)	(55)
M−2ROH	492 (53)	(100)	366 (100)	(63)	372 (100)	(100)	492 (100)	(100)	366 (100)	(100)	372 (100)	(100)
M−2ROH–Me	477 (8)	(12)	351 (10)	(8)	357 (11)	(18)	477 (9)	(9)	351 (11)	(17)	357 (11)	(10)
M−2ROH–C_3H_5	451 (2)	(4)	325 (2)	(2)	331 (2)	(6)	451 (1)	(1)	325 (1)	(2)	331 (1)	(1)
Ion B_2	387 (1)	(1)	261 (1)	(1)	267 (1)	(2)	387 (1)	(1)	261 (1)	(1)	267 (1)	(1)
Ion B_3	373 (12)	(19)	247 (18)	(11)	253 (24)	(26)	373 (20)	(14)	247 (20)	(30)	253 (39)	(21)
Ion C_1	333 (1)	(2)	207 (1)	(1)	213 (4)	(6)	333 (3)	(2)	207 (2)	(2)	213 (5)	(4)
Ion C_2	319 (3)	(2)	193 (3)	(3)	199 (6)	(8)	319 (3)	(2)	193 (2)	(4)	199 (10)	(9)
M−SC–ROH	271 (1)	(1)	271 (2)	(2)	271 (2)	(4)	313 (1)	(0.5)	313 (0.5)	(0.5)	313 (1)	(1)
M−SC–2ROH	253 (4)	(5)	253 (7)	(8)	253 (24)	(26)	253 (10)	(9)	253 (12)	(20)	253 (39)	(21)
SC	239 (31)	(18)	[b]		[b]		239 (6)	(8)	[b]		[b]	
$C_{16}H_{19}$	211 (11)	(9)	211 (5)	(5)	211 (8)	(10)	211 (9)	(9)	211 (9)	(14)	211 (16)	(9)
$C_{11}H_{11}$	143 (38)	(45)	143 (27)	(46)	143 (46)	(52)	143 (47)	(41)	143 (52)	(59)	143 (52)	(38)
C_9H_{11}	119 (34)	(46)	119 (21)	(22)	119 (31)	(40)	119 (45)	(36)	119 (31)	(40)	119 (31)	(20)
C_8H_9	105 (38)	(32)	105 (14)	(30)	105 (27)	(38)	105 (26)	(25)	105 (30)	(38)	105 (27)	(20)
C_4H_9[c]	57 (46)	(19)	57 (91)	(100)	57 (7)	(10)	57 (16)	(28)	57 (36)	(41)	57 (9)	(7)

[a] Mass spectral data are given in the form m/z (relative abundance of 7α isomer) (relative abundance of 7β isomer). Ions B_2, B_3, C_1, and C_2 are defined in Fig. 7.
[b] Exact masses of ions at m/z 113 and 119 did not correspond to C_8H_{17} or $C_8H_{11}D_6$.
[c] The high relative abundances for m/z 57 for sterols lacking fluorine or deuterium suggested partial origination from the side-chain terminus; deuterated sterols did show m/z 63 ions of moderate abundance corresponding to $C_4H_3D_6$, but m/z 183 of the fluorinated sterols corresponded mainly to $C_{14}H_{15}$ (although $C_4F_7H_2$ ions were observed in low abundance).

0.914 (d, 6.6, 3H), 0.694 (s), δ_C (25°C) 171.11, 170.35, 144.17, 122.23, 75.52, 73.20, 55.42, 55.33, 48.04, 42.78, 39.30, 39.28, 37.49, 36.49, 36.46, 36.43, 36.09, 35.65, 28.34, 27.58, 27.47, 25.11, 23.75, 21.63, 21.35, 21.00, 18.96, 18.72, 11.74.

*2.17. [16,16-$^2H_2$]-(25R)-Cholest-5-ene-3β,7α,26-triol triacetate (**18e**) and (25R)-cholest-5-ene-3β,7α,26-triol triacetate (**18d**)*

L-Selectride (2.5 ml, 1 M solution) was added dropwise to a solution of **16e** (120 mg, 0.24 mmol) in anhydrous tetrahydrofuran (30 ml) at −78°C. The reaction mixture was stirred at −78°C for 5 h, warmed to room temperature, and quenched with water (2.5 ml). To this solution was added 6 M sodium hydroxide (2.5 ml) and 30% hydrogen peroxide (2.5 ml). The reaction mixture was stirred at room temperature for 1 h and extracted with chloroform–methanol (10:1). Evaporation of the combined extracts gave a white solid that was dissolved in pyridine (5 ml) and acetic anhydride (0.5 ml) and heated at 100°C for 1 h under nitrogen. After cooling to room temperature, the mixture was poured into ice-water (50 ml) and extracted with dichloromethane (3 × 30 ml). The combined extracts were washed with cold 5% sulfuric acid (3 × 30 ml) and brine (30 ml) and dried over anhydrous sodium sulfate. Evaporation gave a yellow oil that was subjected to MPLC on alumina–$AgNO_3$ (1000 × 10 mm i.d. column, elution with ethyl acetate–hexane 8:92). Evaporation of fractions 20–40 gave **18e** as a colorless oil (115 mg, 88% yield, ~98% purity excluding non-steroidal material): single component by TLC (R_f 0.15, SS-1); high-resolution MS, calcd. for $C_{31}H_{46}D_2O_4$ (M − 60), 486.3678, found 486.3673; MS 486*† (32), 444*† (100), 426*† (35), 411*† (12), 384† (17, $C_{27}H_{40}D_2O$), 367† (4, $C_{27}H_{39}D_2$), 366*† (3), 351*† (4), 307† (7, $C_{20}H_{31}D_2O_2$), 283† (4, $C_{21}H_{27}D_2$), 271† (11, $C_{19}H_{23}D_2O$), 255† (17, $C_{19}H_{23}D_2$), 253† (8, $C_{19}H_{21}D_2$), 239† (5, $C_{18}H_{19}D_2$), 226† (6, $C_{17}H_{18}D_2$), 213† (13, $C_{16}H_{17}D_2$), 211† (13, $C_{16}H_{19}$), 159† (58, $C_{12}H_{15}$), 146† (35, $C_{11}H_{14}$); IR ($CHCl_3$ solution), 2939, 2895, 2872, 1726, 1458, 1438, 1373, 1246, 1112, 1033 cm^{-1}; ^1H and ^{13}C NMR data essentially identical to those of **18d** except for deuterium isotope effects and the absence of signals and couplings for C-16, H-16α, and H-16β.

Similar treatment of **16d** (120 mg) gave (after MPLC on alumina–$AgNO_3$) **18d** (110 mg, 84% yield, ~98% purity) as a colorless oil: single component by TLC (R_f 0.15, SS-1); high-resolution MS, calcd. for $C_{31}H_{48}O_4$ (M − 60), 484.3553, found 484.3536; MS 484*† (3), 442*† (20), 424*† (100), 409*† (9), 364*† (4), 349*† (3), 305† (31, $C_{20}H_{33}O_2$), 281† (2, $C_{21}H_{29}$), 279† (2, $C_{21}H_{27}$), 277† (2, $C_{18}H_{29}O_2$), 269† (1, $C_{19}H_{25}O$), 253† (22, $C_{19}H_{25}$), 251† (13, $C_{19}H_{23}$), 159† (29, $C_{12}H_{15}$), 157† (39, $C_{12}H_{13}$), 143† (63, $C_{11}H_{11}$); IR ($CHCl_3$ solution), 2944, 2870, 1734, 1469, 1373, 1242, 1035, 935 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2.

*2.18. [16,16-$^2H_2$]-(25R)-Cholest-5-ene-3β,7β,26-triol triacetate (**19e**) and (25R)-cholest-5-ene-3β,7β,26-triol triacetate (**19d**)*

Reduction of (**16e**) (120 mg) with lithium aluminum hydride (34 mg) as described for the preparation of **18b**, followed by acetylation and MPLC on alumina–$AgNO_3$ (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 8:92) and evaporation of fractions 55–74, gave **19e** (95 mg, 73% yield, ~97% purity) as a colorless oil: single component by TLC (R_f 0.15, SS-1); high-resolution MS, calcd. for $C_{31}H_{46}D_2O_4$ (M − 60), 486.3678, found 486.3674; MS 486*† (30), 444*† (100), 426*† (38), 411*† (9), 384*† (15), 366*† (3), 351*† (3), 307† (6, $C_{20}H_{31}D_2O_2$), 271† (10, $C_{19}H_{23}D_2O$), 255† (16, $C_{19}H_{23}D_2$), 253† (8, $C_{19}H_{21}D_2$), 226† (5, $C_{17}H_{18}D_2$), 213† (12, $C_{16}H_{17}D_2$), 212† (12, $C_{16}H_{20}$), 211† (11, $C_{16}H_{19}$), 159† (63, $C_{12}H_{15}$), 146† (38, $C_{11}H_{14}$); IR ($CHCl_3$ solution), 2941, 2877, 2854, 1726, 1456, 1375, 1249, 1033 cm^{-1}; ^1H and ^{13}C NMR data essentially identical to those of **19d** except for deuterium isotope effects and the absence of signals and couplings for C-16, H-16α, and H-16β.

Similar treatment of **16d** (120 mg) gave **19d** (50 mg, 38% yield, ~97% purity) as a colorless oil: single component by TLC (R_f 0.15, SS-1); high-resolution MS, calcd. for $C_{31}H_{48}O_4$,

484.3553 (M − 60), found 484.3542; MS, 484*† (8), 442*† (53), 424*† (100), 409*† (11), 364*† (7), 349*† (3), 305† (22, $C_{20}H_{33}O_2$), 269† (2, $C_{19}H_{25}O$), 253† (27, $C_{19}H_{25}$), 251† (13, $C_{19}H_{23}$), 225† (5, $C_{17}H_{21}$), 211† (15, $C_{16}H_{19}$), 199† (12, $C_{15}H_{19}$), 159† (35, $C_{12}H_{15}$), 157† (30, $C_{12}H_{13}$), 143† (46, $C_{11}H_{11}$); IR ($CHCl_3$ solution), 2945, 2872, 1734, 1471, 1456, 1373, 1240, 1034 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2.

*2.19. 25,26,26,26,27,27,27-Heptafluorocholest-5-ene-3β,7α-diol (**20b**), cholest-5-ene-3β,7α-diol (**20a**), [26,26,26,27,27,27-$^2H_6$]cholest-5-ene-3β,7α-diol (**20c**), (25R)-cholest-5-ene-3β,7α,26-triol (**20d**), and [16,16-$^2H_2$]-(25R)-cholest-5-ene-3β,7α,26-triol (**20e**)*

To a solution of **18b** (20 mg, 0.033 mmol) in methanol (10 ml) was added sodium hydroxide (20 mg, 0.5 mmol), and the reaction mixture was stirred at room temperature for a week. TLC analysis showed the disappearance of **18b** and the formation of polar material at R_f 0.32 (SS-6). The reaction mixture was evaporated to a residue that was dissolved in dichloromethane, washed with brine (10 ml), and dried over anhydrous sodium sulfate. Evaporation gave a white solid that was further purified by chromatography (200 × 10 mm i.d. gravity column; 230–400 mesh silica gel; elution with ethyl acetate–hexane 3:7). Evaporation of fractions 6–14 gave **20b** as a white solid (15 mg, 87% yield, ~97% purity): m.p., 212–214°C; single component by TLC (R_f 0.32, SS-6); high-resolution MS, calcd. for $C_{27}H_{39}O_2F_7$, 528.2838, found 528.2830; MS, Table 6; IR, 3483, 3393, 2935, 2858, 1467, 1311, 1244, 1221, 1161, 1055 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4; ^{19}F NMR, δ_F −184.14 (dd of septet, 21.1, 19.9, 6.7 Hz), −76.75 and −76.92 (A_3B_3 portion of A_3B_3X system (Swaminathan et al., 1993)).

Similar treatment of **18a** (50 mg) gave **20a** as a white solid (38 mg, 92% yield, ~99% purity): m.p., 186–188°C (lit. 183–186°C (Kumar et al., 1987), 183–184°C (Kudo et al., 1989), 183–184°C (Lythgoe and Trippett, 1959)); single component by TLC (R_f 0.36, SS-6); MS, Table 6; ^1H and ^{13}C NMR data identical (±0.011 ppm for ^1H, ±0.07 ppm for ^{13}C) to those of **20c** except for the occurrence of signals at δ_H 1.517 (nonet, 6.6 Hz), 0.867 (d, 6.6 Hz, 3H), 0.863 (d, 6.6 Hz, 3H), δ_C 39.49 (replacing 39.34), 27.99 (replacing 27.49), 22.79, 22.55.

Similar treatment of **18c** (16 mg) gave **20c** as a white solid (12 mg, 90% yield, ≥99% purity): m.p., 185–186°C; single component by TLC (R_f 0.36, SS-6); high-resolution MS, calcd. for $C_{27}H_{40}D_6O_2$, 408.3874, found 408.3874; MS, Table 6; IR, 3367, 2933, 2864, 2214, 1458, 1375, 1336, 1259, 1055, 1012, 950 cm^{-1}; NMR, δ_H (50 mM solution) 5.601 (br dd, 5.3, 1.9 Hz), 3.852 (ddd, 5.3, 3.6, 1.7 Hz), 3.580 (tdd, 11.1, 5.1, 4.2 Hz), 2.343 (ddd, 13.1, 5.1, 2.2 Hz), 2.283 (ddt, 13.1, 11.2, 1.9 Hz), 2.003 (br ddd, 12.7, 4.2, 2.9 Hz), 1.705 (dddd, 12.3, 9.7, 6.9, 2.9 Hz), 0.994 (d, 0.6 Hz, 3H), 0.926 (d, 6.6 Hz, 3H), 0.685 (s, 3H), δ_C 146.21, 123.77, 71.21, 65.32, 55.79, 49.36, 42.19, 42.09, 41.97, 39.34, 39.12, 37.45, 37.34, 36.97, 36.13, 35.74, 31.28, 28.24, 27.49, 24.25, 23.67, 20.66, 18.70, 18.22, 11.60 (no signals observed for C-26, C-27).

Similar treatment of **18d** (100 mg) gave compound **20d** (65 mg, 85% yield, ~99% purity) as a white solid: m.p., 203–204°C; single component by TLC (R_f 0.60, SS-7); high-resolution MS, calcd. for $C_{27}H_{46}O_3$, 418.3447, found 418.3434; MS, 418† (5, M^+), 400*† (100), 382*† (8), 367† (4, $C_{26}H_{39}O$), 341† (1, $C_{24}H_{37}O$), 271*† (1), 253*† (2), 225† (6, $C_{17}H_{21}$), 211† (2, $C_{16}H_{19}$), 153† (17, $C_{12}H_9$), 119† (6, C_9H_{11}); IR, 3324, 2965, 2949, 2926, 2905, 2874, 2857, 1458, 1441, 1373, 1364, 1057, 1042, 1013 cm^{-1}; ^1H NMR, δ_H 5.607 (dd, 5.3, 1.7 Hz), 3.853 (m), 3.591 (m), 3.506 (br dd, 10.2, 5.9 Hz), 3.422 (br dd, 10.2, 6.6 Hz), 2.348 (ddd, 13.2, 5.0, 2.0 Hz), 2.286 (ddt, 13.2, 11.2, 2.0 Hz), 0.997 (d, 0.6 Hz, 3H), 0.930 (d, 6.7 Hz, 3H), 0.916 (d, 6.7 Hz, 3H), 0.687 (d, 0.4 Hz, 3H).

Similar treatment of **18e** (100 mg) gave, after MPLC (300 × 10 mm i.d. column, elution with methanol–chloroform 3:97) and evaporation of fractions 19–29, **20e** as a white solid (69 mg, 90% yield, ≥99% purity): m.p., 198–199°C; single component by TLC (R_f 0.60, SS-7); high-resolution MS, calcd. for $C_{27}H_{44}D_2O_3$, 420.3572,

found 420.3583; MS, 420† (2, M^+), 402*† (77), 384*† (100), 369*† (16), 366*† (7), 343† (4, $M-C_3H_9O_2$), 273*† (4), 265† (27, $C_{18}H_{29}D_2O$), 255*† (19), 227† (5, $C_{17}H_{19}D_2$), 211† (14, $C_{16}H_{15}D_2$), 158† (31, $C_{12}H_{10}D_2$), 137 (60); IR, 3314, 2964, 2951, 2928, 2901, 2876, 2854, 1458, 1438, 1376, 1363, 1246, 1051, 1039, 1010 cm^{-1}; ^1H NMR data essentially identical to those of **20d** except for deuterium isotope effects and the absence of signals and couplings for H-16α and H-16β.

*2.20. 25,26,26,26,27,27,27-Heptafluorocholest-5-ene-3β,7β-diol (**21b**), cholest-5-ene-3β,7β-diol (**21a**), [26,26,26,27,27,27-$^2H_6$]cholest-5-ene-3β,7β-diol (**21c**), (25R)-cholest-5-ene-3β,7β,26-triol (**21d**), and [16,16-$^2H_2$]-(25R)-cholest-5-ene-3β,7β,26-triol (**21e**)*

Saponification of **19b** (60 mg) as described for **18b** gave **21b** as a white solid (48 mg, 93% yield, ≥98% purity): m.p., 176–177°C; single component by TLC (R_f 0.36, SS-6); high-resolution MS, calcd. for $C_{27}H_{39}O_2F_7$, 528.2838, found 528.2822; MS, Table 6; IR, 3536, 3362, 2969, 2942, 1468, 1314, 1223, 1157, 1057, 937 cm^{-1}; ^1H and ^{13}C NMR, Tables 3 and 4; ^{19}F NMR, δ_F −184.18 (dd of septet, 21.2, 19.9, 6.7 Hz), −76.73 and −76.94 (A_3B_3 portion of A_3B_3X system (Swaminathan et al., 1993)).

Similar treatment of **19a** (100 mg) gave **21a** as a white solid (72 mg, 87% yield, ≥99% purity): m.p., 177–178°C (lit. 174–178°C (Kumar et al., 1987), 180.5–181.0°C (Kudo et al., 1989), 172–175°C (Smith and Price, 1967), 176–182°C (Ruzicka et al., 1944)); single component by TLC (R_f 0.38, SS-6); MS, Table 6; ^1H and ^{13}C NMR data identical (±0.007 ppm for ^1H, ± 0.02 ppm for ^{13}C) to those of **21c** except for the occurrence of signals at δ_H 1.519 (nonet, 6.6 Hz), 0.869 (d, 6.6 Hz, 3H), 0.864 (d, 6.6 Hz, 3H), δ_C 39.46 (replacing 39.33), 27.99 (replacing 27.49), 22.80, 22.54.

Similar treatment of **19c** (60 mg) gave **21c** as a white solid (45 mg, 90% yield, >99% purity): m.p., 176–178°C; single component by TLC (R_f 0.38, SS-6); high-resolution MS, calcd. for $C_{27}H_{40}D_6O_2$, 408.3874, found 408.3897; MS, Table 6; IR, 3331, 2935, 2903, 2864, 2850, 2214, 1464, 1375, 1354, 1302, 1136, 1055, 1014, 947 cm^{-1}; NMR, δ_H (50 mM solution) 5.289 (td, 2.0, 0.5 Hz), 3.843 (dt, 7.9, 2.3 Hz), 3.544 (tdd, 11.3, 5.0, 4.1 Hz), 2.331 (ddd, 13.2, 4.9, 2.2 Hz), 2.253 (ddt, 13.3, 11.4, 2.1 Hz), 2.021 (br ddd, 12.7, 4.0, 2.9 Hz), 1.804 (dddd, 12.3, 9.7, 7.2, 2.6 Hz), 1.050 (d, 0.5 Hz, 3H), 0.922 (d, 6.6 Hz, 3H), 0.694 (d, 0.5 Hz, 3H), δ_C 143.43, 125.40, 73.31, 71.36, 55.91, 55.40, 48.20, 42.88, 41.68, 40.83, 39.51, 39.33, 36.89, 36.39, 36.17, 35.70, 31.51, 28.52, 27.49, 26.35, 23.79, 21.04, 19.13, 18.74, 11.79 (no signals observed for C-26, C-27).

Similar treatment of **19d** (50 mg) gave compound **21d** (30 mg, 78% yield, ~99% purity) as a white solid: m.p., 198–199°C; single component by TLC (R_f 0.60, SS-7); high-resolution MS, calcd. for $C_{27}H_{44}O_2$ (M − 18), 400.3341, found 400.3342; MS, 418† (7, M^+), 400*† (100), 382*† (51), 367*† (13), 341† (5, $C_{24}H_{37}O$), 271*† (3), 263† (10, $C_{18}H_{31}O$), 253*† (10), 225† (3, $C_{17}H_{21}$), 211† (10, $C_{16}H_{19}$), 157† (28, $C_{12}H_{13}$), 143† (48, $C_{11}H_{11}$), 119† (35, C_9H_{11}); IR, 3306, 2964, 2926, 2886, 1456, 1437, 1417, 1398, 1375, 1356, 1064, 1041, 1020, 976 cm^{-1}; ^1H NMR, δ_H 5.294 (t, 2.0 Hz), 3.846 (ddt, 8.0, 6.0, 2.3 Hz), 3.552 (m), 3.504 (br dt, ~10.8, ~6 Hz), 3.427 (br dt, ~10.4, ~6 Hz), 2.337 (ddd, 13.3, 5.0, 2.2 Hz), 2.257 (ddt, 13.3, 11.1, 2.1 Hz), 1.052 (s, 3H), 0.926 (d, 6.6 Hz, 3H), 0.917 (d, 6.7 Hz, 3H), 0.696 (d, 0.4 Hz, 3H).

Similar treatment of **19e** (80 mg) gave compound **21e** (58 mg, 94% yield, 99% purity) as a white solid: m.p., 195–196°C, single component by TLC (R_f 0.60, SS-7); high-resolution MS, calcd. for $C_{27}H_{44}D_2O_3$, 420.3572, found 420.3549; MS, 420† (4, M^+), 402*† (100), 384*† (31), 369*† (14), 366*† (4), 343† (6, $M-C_3H_9O_2$), 273*† (6), 265† (9, $C_{18}H_{29}D_2O$), 255† (11), 227† (3, $C_{17}H_{19}D_2$), 211† (8, $C_{16}H_{15}D_2$), 158† (14, $C_{12}H_{10}D_2$), 137 (21); IR, 3313, 3225, 2964, 2928, 2885, 2864, 1460, 1373, 1168, 1138, 1047, 1014, 980 cm^{-1}; ^1H NMR data essentially identical to those of **21d** except for deuterium isotope effects and the absence of signals and couplings for H-16α and H-16β.

*2.21. (25R)-26-Hydroxycholest-4-en-3-one (**22d**), [16,16-$^2H_2$]-(25R)-26-hydroxycholest-4-en-3-one (**22e**), 3:1 mixture of (25R)-26-hydroxycholest-4-ene-3,7-dione (**23d**) and (25R)-26-hydroxycholest-5-ene-3,7-dione, (25R)-7α,26-dihydroxycholest-4-en-3-one (**24d**), [16,16-$^2H_2$]-(25R)-7α,26-dihydroxycholest-4-en-3-one (**24e**), (25R)-7β,26-dihydroxycholest-4-en-3-one (**25d**), and [16,16-$^2H_2$]-(25R)-7β,26-dihydroxycholest-4-en-3-one (**25e**)*

A solution of **20e** (30 mg) in butyl acetate (6 ml) was added to a TES buffer solution (6 ml, 50 mM, pH 7.5) containing cholesterol oxidase from *Streptomyces* sp. (50 units) and catalase (40 000 units). The two-phase mixture (Lee and Biellmann, 1988) was stirred in a vial at ca. 22°C with a magnetic stirrer and occasionally shaken vigorously by hand. After 30 h, TLC analysis indicated complete disappearance of **20e** and formation of a less polar product (R_f 0.70, SS-7). The reaction mixture was extracted with 10% methanol–chloroform (2 × 20 ml), and the organic extracts were washed with water and brine and dried over anhydrous sodium sulfate. Evaporation gave a white solid that was subjected to MPLC (300 × 10 mm i.d. column; elution with methanol–chloroform 3:97). Evaporation of fractions 36–48 gave **24e** as a white solid (25 mg, 86% yield, 99% purity): m.p., 173–174°C; single component by TLC (R_f 0.70, SS-7); high-resolution MS, calcd. for $C_{27}H_{42}D_2O_3$, 418.3416, found 418.3415; MS 418† (11, M^+), 400*† (22), 390*† (7), 385*† (6), 362† (22, $M-C_3H_4O$), 271*† (8), 265† (4, $C_{18}H_{29}D_2O$), 227† (6, $C_{16}H_{15}D_2O$), 215† (4, $C_{15}H_{15}D_2O$), 187† (6, $C_{13}H_{11}D_2O$), 174† (12, $C_{12}H_{10}D_2O$); IR, 3338, 2962, 2930, 2885, 2862, 1664, 1618, 1458, 1375, 1043, 1031, 1007 cm^{-1}; ^1H NMR data essentially identical to those of **24d** except for deuterium isotope effects and the absence of signals and couplings for H-16α and H-16β.

Similar treatment of **1d** (25 mg) gave **22d** (22 mg, 83% yield, 98% purity (contains 1% **1d**)) as a white solid: m.p., 132–134°C; single component by TLC (R_f 0.85, SS-7); high-resolution MS, calcd. for $C_{27}H_{44}O_2$, 400.3341, found 400.3326; MS, 400† (80, M^+), 385*† (8), 382*† (6), 367*† (3), 358† (22, $M-C_2H_2O$), 343† (7, $M-C_3H_5O$), 315† (5), 277 † (26, $M-C_8H_{11}O$), 271*† (12), 263† (5, $C_{18}H_{31}O$), 253*† (3), 229† (54, $C_{16}H_{21}O$ and $C_{17}H_{25}$), 211† (8, $C_{16}H_{19}$), 149† (12, $C_{11}H_{17}$), 147† (25, $C_{11}H_{15}$), 124† (100, $C_8H_{12}O$); IR, 3431, 2931, 2870, 2856, 1660, 1612, 1465, 1458, 1448, 1433, 1377, 1361, 1230, 1043 cm^{-1}; ^1H NMR, δ_H 5.724 (m), ~3.503 (br dd, ~10.5, 6 Hz), ~3.426 (br dd, ~10.5, 6 Hz), 1.181 (d, 0.6 Hz, 3H), 0.915 (d, 6.7 Hz, 6H), 0.709 (d, 0.6 Hz, 3H).

Similar treatment of **1e** (23 mg) gave **22e** (19 mg, 83% yield, ≥99% purity) as a white solid: m.p., 134–135°C; single component by TLC (R_f 0.85, SS-7); high-resolution MS, calcd. for $C_{27}H_{42}D_2O_2$, 402.3467, found 402.3486; MS, 402† (70, M^+), 387*† (8), 384*† (8), 369*† (4), 360† (20, $M-C_2H_2O$), 345† (6, $M-C_3H_5O$), 317 † (5, $C_{22}H_{33}D_2O$), 301 (3), 279† (27, $M-C_8H_{11}O$), 273*† (11), 265† (5, $C_{18}H_{29}D_2O$), 255*† (3), 229† (16, $C_{16}H_{21}O$), 211† (10, $C_{16}H_{19}$), 149† (25, $C_{11}H_{17}$ and $C_{10}H_{13}O$), 147† (10, $C_{11}H_{15}$), 124† (100, $C_8H_{12}O$); IR, 3433, 2931, 2862, 1664, 1616, 1465, 1458, 1437, 1377, 1338, 1236, 1039 cm^{-1}; ^1H and ^{13}C NMR data essentially identical to those of **22d** except for deuterium isotope effects and the absence of signals and couplings for C-16, H-16α, and H-16β.

Similar treatment of **17d** (40 mg; 48 h reaction time) gave mainly recovered starting material, but a 3:1 mixture of **23d** and (25*R*)-26-hydroxycholest-5-ene-3,7-dione was isolated as a pale yellow solid (4 mg, 9% yield): two components by TLC (R_f 0.80 and 0.83, SS-7); high-resolution MS, calcd. for $C_{27}H_{44}O_3$, 414.3134, found 414.3138; MS, 414† (100, M^+), 399*† (5), 396*† (11), 386† (3, $M-CO$), 381*† (3), 291† (7, $C_{19}H_{31}O_2$), 285*† (34), 245† (18, $C_{16}H_{21}O_2$), 203† (20, $C_{16}H_{11}$), 190† (47, $C_{12}H_{14}O_2$), 124† (16, $C_8H_{12}O_2$); ^1H NMR (**23d**), δ_H 5.709 (dt, 2.5, 0.8 Hz), 3.503 (dd, 10.5, 5.9 Hz), 3.461 (ddd, 15.1, 2.5, 1.0, H-6β), 3.426 (dd, 10.5, 6.5 Hz), 3.051 (dd, 15.0, 0.7 Hz, H-6α), 1.419 (d, 0.7 Hz, 3H), 0.925 (d, 6.7 Hz, 3H), 0.915 (d, 6.7 Hz, 3H), 0.709 (d, 0.5 Hz, 3H); ^1H NMR (Δ^5 isomer of **23d**), δ_H 5.676 (dt, 2.4, 0.7 Hz), 3.505 (dd, 10.5, 5.9 Hz), 3.426 (dd, 10.5, 6.5 Hz), 3.424 (ddd, 17.5, 2.4, 0.6, H-4β), 3.076 (ddd, 17.5, 2.2, 0.6 Hz, H-4α), 2.573 (ddd, 15.9, 13.2, 5.8 Hz), ~2.426 (m, H-2α), 2.41 (m, H-15α), 2.309 (dd, 11.9, 10.8 Hz, H-8β), 2.142 (ddd, 13.8, 5.8, 4.0

Hz, H-1β), 1.362 (d, 0.4 Hz, 3H), 0.934 (d, 6.7 Hz, 3H), 0.917 (d, 6.7 Hz, 3H), 0.717 (d, 0.5 Hz, 3H); partial ^{13}C NMR (Δ^5 isomer of **23d**; data from HSQC spectrum), 54.75 (C-17), 47.4 (C-4), 45.3 (C-8), 37.0 (C-2), 35.0 (C-1), 26.2 (C-15), 18.7 (C-21), 17.2 (C-19), 12.0 (C-18). Similar reaction of **17e** also gave mainly recovered starting material, with a small amount of a 3:1 mixture of the deuterated analogs of **23d** and its Δ^5 isomer.

Similar treatment of **20d** (60 mg) gave **24d** (54 mg, 90% yield, 99% purity) as a white solid: m.p., 175–176°C (lit. 168–169°C (Kim et al., 1997)); single component by TLC (R_f 0.70, SS-7); high-resolution MS, calcd. for $C_{27}H_{44}O_3$, 416.3290, found 416.3271; MS, 416† (13, M^+), 401*† (1), 398*† (24), 383*† (3), 380*† (1), 360† (18, $C_{24}H_{40}O_2$), 269*† (7), 263† (3, $C_{18}H_{31}O$), 245† (2, $C_{16}H_{21}O_2$) 145† (8, $C_{11}H_{13}$), 124† (100, $C_8H_{12}O$), 109† (11, C_7H_9O); IR, 3350, 2932, 2886, 2860, 1665, 1464, 1375, 1276, 1188, 1049, 1005, 949 cm^{-1}; ^{1}H NMR, δ_H 5.805 (m), 3.969 (m), 3.501 (dd, 10.5, 5.9 Hz), 3.426 (dd, 10.5, 6.5 Hz), 1.193 (d, 0.7 Hz, 3H), 0.922 (d, 6.6 Hz, 3H), 0.915 (d, 6.7 Hz, 3H), 0.717 (d, 0.5 Hz, 3H).

Similar treatment of **21d** (28 mg) gave **25d** (26 mg, 94% yield, $\geq$99% purity) as a white solid: m.p., 158–159°C; single component by TLC (R_f 0.75, SS-7); high-resolution MS, calcd. for $C_{27}H_{44}O_3$, 416.3290, found 416.3297; MS, 416† (7, M^+), 398*† (100), 388*† (1), 383*† (7), 380*† (9), 365*† (4), 360† (3, $C_{24}H_{40}O_2$), 287*† (1), 269*† (34), 263† (19, $C_{18}H_{31}O$), 255† (6, $C_{18}H_{23}O$), 245† (6, $C_{16}H_{21}O_2$), 227† (15, $C_{16}H_{19}O$ and $C_{17}H_{23}$), 213 (11), 175† (34, $C_{12}H_{15}O$), 161† (36, $C_{11}H_{13}O$ and $C_{12}H_{17}$), 136† (54, $C_9H_{12}O$), 95† (70, $C_7H_{11}O$ and C_6H_7O); IR, 3418, 2938, 2866, 1655, 1465, 1373, 1232, 1039, 947 cm^{-1}; ^{1}H NMR, δ_H 5.761 (m), 3.501 (dd, 10.5, 6.5 Hz), 3.460 (ddd, 11.2, 9.2, 5.2 Hz), 3.428 (dd, 10.5, 5.9 Hz), 1.210 (d, 0.6 Hz, 3H), 0.929 (d, 6.7 Hz, 3H), 0.916 (d, 6.7 Hz, 3H), 0.734 (d, 0.6 Hz, 3H).

Similar treatment of **21e** (29 mg) gave **25e** (25 mg, 85% yield, ~98% purity) as a white solid: m.p., 156–157°C; single component by TLC (R_f 0.75, SS-7); high-resolution MS, calcd. for $C_{27}H_{42}D_2O_3$, 418.3416, found 418.3426; MS, 418† (52, M^+), 400*† (70), 390*† (5), 385*† (8), 382*† (5), 362† (14, $M-C_3H_4O$), 356† (9, $C_2H_6O_2$), 289*† (2), 271*† (21), 265† (10, $C_{18}H_{29}D_2O$), 257† (3, $C_{18}H_{25}O$), 238† (5, $C_{16}H_{26}D_2O$), 228† (8, $C_{14}H_{27}O_2$), 213 (5), 152† (17, $C_9H_{12}O_2$), 124† (100, $C_8H_{12}O$); IR, 3445, 2935, 2854, 1647, 1637, 1608, 1456, 1419, 1373, 1338, 1278, 1232, 1195, 1060, 1043, 945 cm^{-1}; ^{1}H NMR data essentially identical to those of **25d** except for deuterium isotope effects and the absence of signals and couplings for H-16α and H-16β.

*2.22. 5-Bromo-6β,19-epoxy-5α-cholestan-3β-ol acetate (**27a**), 5-bromo-6β,19-epoxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-3β-ol acetate (**27b**), and [26,26,26,27,27,27-$^2H_6$]5-bromo-6β,19-epoxy-5α-cholestan-3β-ol acetate (**27c**)*

To a solution of acetate **2a** (2.15 g, 5.0 mmol) in dioxane (30 ml) were added water (2.5 ml) and perchloric acid (70%, five drops), followed by cooling to 0°C and addition of *N*-bromoacetamide (0.70 g, 5.1 mmol) in one portion. The reaction mixture was stirred at 0°C in the dark for 1 h, warmed to room temperature, and stirred for another 12 h in the dark. Crude 5-bromo-5α-cholestane-3β,6β-diol 3-acetate (**26a**) was precipitated by addition of water (300 ml). The white precipitate (2.2 g) was collected by filtration, washed with water, dried in vacuo, and used without further purification. Similar reaction of **2a** (1.0 g) gave crude **26a** showing by TLC (SS-4) and ^{1}H NMR a 6:3:1 mixture of **26a** (R_f 0.65), 6β-bromo-5α-cholestane-3β,5-diol 3-acetate (R_f 0.77), and unreacted **2a** (R_f 0.95). Trituration with ethyl acetate (6 ml) followed by recrystallization from hexane–acetone (95:5, ~20 ml) furnished an analytical sample of **26a** (0.20 g, 99% purity, containing 1% 6β-bromo-5α-cholestane-3β,5-diol 3-acetate): m.p.; 167–168°C (lit. 168–169°C (Kalvoda et al., 1963)); MS, 524*§ (0.8, M^+), 506*§ (0.5), 491*§ (0.6), 444* (31), 429* (8), 426* (13), 411* (2), 385* (68), 384* (100), 369* (36), 366* (45), 356 (14), 351* (10), 331* (26), 271* (17), 253* (15), 247 (12), 244 (12), 229 (23), 211 (19), 107 (40); ^{1}H and ^{13}C NMR, Tables 1 and 2.

To a suspension of calcium carbonate (4.0 g) and lead tetraacetate (15.0 g, dried overnight in a desiccator over NaOH) in cyclohexane (300 ml) at

80°C were added crude **26a** (2.0 g) and iodine (2.0 g) in one portion. The reaction mixture was stirred vigorously under reflux with irradiation from a 500 watt halogen utility lamp. After about 1.5 h, when the deep red solution had become colorless, the reaction mixture was cooled to room temperature and the solid material was removed by filtration. The filtrate was washed with saturated sodium thiosulfate and brine and evaporated to a pale yellow oil, which was subjected to MPLC (300 × 25 mm i.d. column; elution with ethyl acetate–hexane 4:96). Evaporation of fractions 18–32 gave a clean 3:1 mixture of **27a** and epoxide **4a** (1.4 g, 53% crude yield). Further purification of a portion (~10 mg) of this mixture on MPLC (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 4:96), followed by evaporation of fractions 19–23 gave an analytical sample of **27a** (8 mg, 95% purity): m.p., 143–144°C (lit. 143–144°C (Kalvoda et al., 1963)); single component by TLC (R_f 0.32, SS-1); MS, 522*[§] (0.4, M − H_2O), 462*[§] (0.3), 443* (16), 383* (100), 365* (7), 353 (18), 289 (8), 247 (1), 253* (1), 243 (13), 229 (18), 211 (11), 143 (17), 121 (23); ^1H and ^{13}C NMR, Tables 1 and 2.

Similar treatment of **2b** (425 mg) gave crude **27b** (300 mg, 60% yield), which was used without further purification.

Similar treatment of **2c** (140 mg) gave crude **27c** (90 mg, 53% yield). Further purification of a portion (20 mg) of this mixture on MPLC (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 4:96), followed by evaporation of fractions 28–31 gave an analytical sample of **27c** (14 mg, ~90% purity (containing 5% **4c**)): m.p., 142–143°C; single component by TLC (R_f 0.32, SS-6); high-resolution MS, calcd. for $C_{29}H_{43}D_6O_4^{79}Br$ (M − 18), 528.3085, found 528.3069; MS, 528*[†§] (0.3), 468*[†§] (0.3), 449*[†] (13), 389*[†] (100), 371*[†] (7), 359[†] (18, $C_{26}H_{35}D_6$), 289[†] (7, $C_{18}H_{25}O_3$), 253[†] (3, $C_{18}H_{25}D_6$ and $C_{19}H_{25}$), 243[†] (12, $C_{17}H_{23}O$), 229[†] (20, $C_{16}H_{21}O$), 211[†] (9, $C_{16}H_{19}$), 143[†] (14, $C_{11}H_{11}$); IR, 2940, 2866, 2212, 1736, 1445, 1371, 1263, 1036 cm^{-1}; ^1H and ^{13}C NMR data identical (±0.002 ppm for ^1H, ±0.04 ppm for ^{13}C) to those of **27a** except for δ_C 39.32 (replacing 39.47) and 27.48 (replacing 27.99) and the absence of signals corresponding to H-26, H-27, C-26, and C-27.

*2.23. 25,26,26,26,27,27,27-Heptafluorocholest-5-ene-3β,19-diol 3-acetate (**28b**), cholest-5-ene-3β,19-diol 3-acetate (**28a**), and [26,26,26,27,27,27-$^2H_6$]cholest-5-ene-3β,19-diol 3-acetate (**28c**)*

Zinc dust (1.5 g) was added to a solution of crude **27b** (250 mg) in absolute ethanol (50 ml), and the reaction mixture was heated at reflux for 6 h. TLC analysis (SS-3) showed the disappearance of **27b** and formation of polar material at R_f 0.36. Unreacted zinc dust was removed by filtration, and the filtrate was evaporated to an oil, which was subjected to MPLC (1000 × 10 mm i.d. column; elution with ethyl acetate–hexane 6:94). Evaporation of fractions 34–40 gave **28b** as a white solid (120 mg, 55% yield, ~98% purity): m.p., 134–135°C; single component by TLC (R_f 0.36, SS-3); high-resolution MS, calcd. for $C_{27}H_{37}OF_7$ (M − 60), 510.2734, found 510.2733; MS, 570[†] (0.1, M^+), 552*[†] (0.3), 510*[†] (19), 492*[†] (10), 480* (67), 479*[†] (100), 478*[†] (25), 375[†] (15, $C_{18}H_{26}F_7$), 373[†] (22, $C_{18}H_{24}F_7$), 333[†] (8, M − $C_{14}H_{21}O_3$), 319[†] (8, $C_{14}H_{18}F_7$), 291[†] (5, $C_{12}H_{14}F_7$), 265[†] (6, $C_{10}H_{12}F_7$), 253*[†] (4), 241[†] (8, $C_{18}H_{25}$), 239[†] (8, SC), 227[†] (5, $C_{17}H_{23}$), 213[†] (7, $C_{16}H_{21}$), 211[†] (7, $C_{16}H_{19}$), 145[†] (51, $C_{11}H_{13}$); IR, 3601, 2943, 2893, 1732, 1709, 1474, 1441, 1381, 1317, 1238, 1159, 1034 cm^{-1}; ^1H and ^{13}C NMR, Tables 1 and 2.

Similar treatment of **27a** (1.0 g) gave **28a** as a white solid (0.63 g, 74% yield, 99% purity): m.p., 120–121°C (lit. 119–120°C (Kalvoda et al., 1963)); single component by TLC (R_f 0.38, SS-3); MS, 426 (0.5, M − 18), 384* (19), 366* (14), 354* (68), 353* (100), 352* (19), 253* (5), 249 (6), 247 (14), 241 (12), 227 (8), 213 (7), 211 (7), 207 (3), 145 (45); ^1H and ^{13}C NMR data identical (± 0.002 ppm for ^1H, ±0.04 ppm for ^{13}C) to those of **28c** except for the occurrence of signals at δ_H 1.514 (nonet, 6.6 Hz), 0.865 (d, 6.6 Hz, 3H), 0.860 (d, 6.6 Hz, 3H), δ_C 39.47 (replacing 39.35), 27.98 (replacing 27.50), 22.80, 22.54. Early fractions from the purification of **28a** contained the α-epox-

ide acetate **4a**, which showed ^{1}H and ^{13}C NMR data essentially identical to those in Tables 1 and 2.

Similar treatment of **27c** (75 mg) gave **28c** as a white solid (39 mg, 52% yield, >99% purity): m.p., 118–119°C; single component by TLC (R_f 0.38, SS-3); high-resolution MS, calcd. for $C_{27}H_{38}D_6O$ (M − 60), 390.3769, found 390.3781; MS, 432*† (0.4), 390*† (11), 372*† (10), 360* (55), 359*† (100), 358*† (18), 255† (2, $C_{18}H_{27}D_6$), 253† (10, $C_{18}H_{25}D_6$ and $C_{19}H_{25}$), 241† (7, $C_{18}H_{25}$), 227† (4, $C_{17}H_{23}$), 213† (5, $C_{15}H_{21}D_6$), 211† (6, $C_{16}H_{19}$), 145† (33, $C_{11}H_{13}$); IR, 3491, 2940, 2868, 2214, 1768, 1703, 1467, 1448, 1381, 1265, 1038 cm^{-1}; NMR, δ_H (50 mM solution) 5.772 (dt, 5.2, 2.1 Hz), 4.644 (tt, 11.5, 4.8 Hz), 3.828 (dd, 11.4, 0.6 Hz), 3.623 (br d, 11.4 Hz), 2.417 (ddd, 13.1, 4.9, 2.3 Hz), 2.267 (ddddd, 12.9, 11.5, 3.3, 2.7, 1.9 Hz), 2.029 (s, 3H), 1.957 (ddd, 14.0, 4.1, 3.2 Hz), 1.618 (br qd, 13.2, 4.0 Hz), 1.494 (dddd, 14.2, 12.9, 11.5, 4.1 Hz), 1.258 (dddd, ~13, 11.5, 9.5, 3.0 Hz), 0.911 (d, 6.5 Hz, 3H), 0.728 (d, 0.4 Hz, 3H), δ_C (25°C) 170.49, 134.50, 128.30, 73.39, 62.70, 57.55, 56.08, 50.29, 42.50, 41.58, 39.98, 39.35, 38.20, 36.16, 35.76, 33.35, 33.07, 31.22, 28.21, 28.09, 27.50, 24.06, 23.80, 21.75, 21.37, 18.68, 12.19 (no signals observed for C-26, C-27).

*2.24. 25,26,26,26,27,27,27-Heptafluorocholest-5-ene-3β,19-diol (**29b**), cholest-5-ene-3β,19-diol (**29a**), and [26,26,26,27,27,27-$^2H_6$]cholest-5-ene-3β,19-diol (**29c**)*

To a solution of **28b** (100 mg, 0.175 mmol) in methanol (20 ml) was added sodium carbonate (100 mg, 1.0 mmol), the reaction mixture was stirred at room temperature overnight. TLC analysis (SS-6) showed the disappearance of **28b** and the formation of polar material at R_f 0.30. The solvent was evaporated to a residue that was dissolved in dichloromethane, washed with brine (10 ml), and dried over anhydrous sodium sulfate. Evaporation gave a white solid that was subjected to MPLC (200 × 10 mm i.d. column, elution with ethyl acetate–hexane 2:8). Evaporation of fractions 31–47 gave **29b** as a white solid (78 mg, 84% yield, 98% purity): m.p., 188–189°C; single component by TLC (R_f 0.30, SS-6); high-resolution MS, calcd. for $C_{27}H_{39}O_2F_7$, 528.2838, found 528.2829; MS, 528† (0.4, M^+), 510*† (25), 498*† (15), 497*† (13), 496*† (5), 492*† (4), 480*† (100), 479*† (41), 478*† (4), 465† (3, $M - C_2H_7O_2$), 437† (3, $M - C_4H_{11}O_2$), 373† (22, $M - C_9H_{15}O_2$), 319† (4, $M - C_{13}H_{21}O_2$), 265† (3, $C_{10}H_{12}F_7$), 253*† (1), 241† (9, $C_{18}H_{25}$), 239† (11, SC), 211† (5, $C_{16}H_{19}$), 145† (40, $C_{11}H_{13}$), 131 (27, $C_{10}H_{11}$); IR, 3405, 3293, 2944, 2907, 2872, 2851, 1474, 1445, 1314, 1223, 1159, 1038 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; ^{19}F NMR, δ_F −184.16 (dd of septet, 21.3, 19.9, 6.6 Hz), −76.73 and −76.95 (A_3B_3 portion of A_3B_3X system (Swaminathan et al., 1993)).

Similar treatment of **28a** (200 mg) gave **29a** as a white solid (158 mg, 87% yield, 99% purity): m.p., 160–162°C (lit. 162–164°C (Kalvoda et al., 1963)); single component by TLC (R_f 0.34, SS-6); MS, 402 (0.8, M^+), 384* (30), 372* (23), 371* (18), 370* (8), 366* (7), 354* (100), 353* (42), 352* (6), 339 (5), 311 (2), 253* (2), 247 (22), 241 (18), 211 (4), 145 (30), 131 (19); ^{1}H and ^{13}C NMR data identical (±0.006 ppm for ^{1}H, ±0.04 ppm for ^{13}C) to those of **29c** except for the occurrence of signals at δ_H 1.514 (nonet, 6.6 Hz), 0.865 (d, 6.6 Hz, 3H), 0.860 (d, 6.6 Hz, 3H), δ_C 39.47 (replacing 39.35), 27.99 (replacing 27.49), 22.80, 22.54.

Similar treatment of **28c** (30 mg) gave **29c** as a white solid (23 mg, 85% yield, 99% purity): m.p., 159–161°C; single component by TLC (R_f 0.26, SS-5); high-resolution MS, calcd. for $C_{27}H_{40}D_6O_2$, 408.3874, found 408.3897; MS, 408† (0.8, M^+), 390*† (21), 378*† (44), 377*† (19), 376*† (20), 372*† (8), 360*† (100), 359*† (46), 358*† (18), 345† (5, $M - C_2H_7O_2$), 317† (1, $M - C_4H_{11}O_2$), 259*† (6), 253† (22, $M - C_9H_{15}O_2$ and $C_{19}H_{25}$), 241† (22, $C_{18}H_{25}$), 211† (7, $C_{16}H_{19}$), 199† (23, $M - C_{13}H_{21}O_2$), 145† (52, $C_{11}H_{13}$), 131† (37, $C_{10}H_{11}$); IR, 3402, 2934, 2866, 2213, 1467, 1442, 1377, 1333, 1051 cm^{-1}; NMR, δ_H (50 mM solution) 5.744 (dt, 5.3, 2.0 Hz), 3.817 (dd, 11.4, 0.7 Hz), 3.607 (d, 11.4 Hz), 3.570 (tt, 11.2, 4.7 Hz), 2.379 (ddd, 13.0, 4.7, 2.4 Hz), 2.189 (ddddd, 13.1, 11.3, 3.3, 2.6, 2.0 Hz), 2.046 (br dt, ~12.9, 3.6 Hz), 2.029 (dtd, 17.9, 5.3, 2.6 Hz), 1.937

(ddd, 13.8, 4.0, 3.0 Hz), 1.625 (br qd, 13.2, 3.9 Hz), 1.403 (dddd, 14.2, 12.9, 11.3, 4.1 Hz), 1.260 (dddd, 13.3, 11.5, 9.5, 3.1 Hz), 0.911 (d, 6.6 Hz, 3H), 0.730 (d, 0.5 Hz, 3H), δ_C (25°C) 135.52, 127.37, 71.34, 62.71, 57.64, 56.10, 50.37, 42.51, 42.30, 41.50, 40.02, 39.35, 36.16, 35.75, 33.39, 33.32, 31.95, 31.23, 28.21, 27.49, 24.06, 23.80, 21.79, 18.68, 12.20 (no signals observed for C-26, C-27).

*2.25. [26,26,26-$^2H_3$]-(25RS)-cholest-5-ene-3β, 25-diol (**1g** and **1h**)*

A solution of CD_3I (160 mg, 1 mmol) in diethyl ether (2 ml) was added dropwise, followed by stirring at room temperature for 2 h. After removal of excess magnesium turnings, the reaction mixture was poured into water, extracted with diethyl ether (20 ml), and washed with brine (10 ml). The organic extracts were dried over anhydrous sodium sulfate and evaporated to a residue that was purified by MPLC (elution with ethyl acetate–hexane 15:85) to give a 1:1 mixture of C-25 epimers **1g** and **1h** as a white solid (85 mg, 95% yield, ~96% purity): m.p.: 178–180°C, single component by TLC (R_f 0.20, ethyl acetate–hexane 1:3); high-resolution MS, calcd. for $C_{27}H_{43}D_3O_2$, 405.3686, found 405.3681; MS (98% d_3, 2% d_2), 405† (34, M^+), 390* (8), 387*† (96), 372*† (46), 369* (27), 354* (28), 302 (13), 300† (18, $C_{21}H_{32}O$), 299† (14, $C_{21}H_{31}O$), 276† (37, $C_{20}H_{30}D_3$), 273*† (20), 271† (70, M − SC–2), 255*† (26, $C_{19}H_{27}$), 253† (16, M − H_2O–SC–2), 231† (16, $C_{16}H_{23}O$), 213† (38, $C_{21}H_{16}$), 145† (55, $C_{11}H_{13}$), 114† (9, SC–H_2O), 62† (100, $C_3H_4D_3O$); IR, 3250, 2935, 2862, 2220, 1469, 1375, 1230, 1195, 1136, 1055, 956, 920 cm^{-1}; NMR data matched chemical shifts reported[2] (Wilson et al., 1994) for the undeuterated sterol **1f** to ±0.002 ppm for ^1H (except H-3α) and ±0.02 ppm for ^{13}C except for deuterium isotope effects at the end of the side chain[4].

*2.26. Stereochemical NMR assignments of the C-19 protons of **27a–c**, **28a–c**, and **29a–c***

Stereochemical assignments of the diastereotopic C-19 protons of 6β,19-ether **27a** and 19-hydroxycholesteryl acetate (**28a**) were determined from 1D NOE difference experiments on non-degassed 50–100 mM samples (1 s weak irradiation at a single frequency; 90° pulse; 2.7 s acquisition time). For **27a**, irradiation of H-8β (δ_H 1.61) led to enhancement of the upfield H-19 signal (δ_H 3.745), and irradiation of H-4β (δ_H 2.27) resulted in enhanced signals at δ_H 3.922 (dd, 8.3, 1.3 Hz) and 4.06 (d, 4.5 Hz, 3-OH)[5]. These observations demonstrate that the downfield C-19 proton of **27a** is positioned over ring A (pro-*R*). For **28a**, irradiation of the downfield C-19 proton (δ_H 3.83) led to enhancements of H-2β (strong), H-4β (strong), and H-8β (weak), whereas irradiation of the upfield C-19 proton (δ_H 3.62) gave enhancements for H-1β (weak), H-4β (weak), and H-11β (very strong). Also, the downfield H-19 signal was enhanced by irradiation of H-2β (strong), H-4β (strong), and H-11β (weak), whereas the upfield H-19 signal was enhanced by irradiation of H-1β (weak), H-4β (weak), and H-11β (strong). These observations are compatible only with a predominant conformation having oxygen over ring B, with the downfield C-19 proton positioned over ring A (pro-*R*). Weaker NOEs correspond to a minor conformer with oxygen positioned between C-1 and C-11. The major and minor conformer are predicted by molecular mechanics (MMX and MM3 force fields) to be similar in energy but ca. 1.5 kcal/mol lower in energy than the rotamer with oxygen over ring A. Based on these results, stereochemical assignments for the C-19 protons of **27b–c**, **28b–c**, and **29a–c** were made by chemical shift comparisons.

[4] The ^{13}C NMR spectrum showed two terminal methyl singlets of roughly equal but diminished intensity, and the ^1H NMR spectrum showed two singlets of equal intensity at δ_H 1.208 and 1.210 (1.214 and 1.215 for the undeuterated sterol). The HSQC spectrum showed correlations between the downfield proton and the downfield carbon and between the upfield proton and upfield carbon, but assignments of the signals to individual C-25 epimers could not be established.

[5] Approximately one-third of the ^1H NMR oxysterol spectra showed a ca. 4–5 Hz coupling of H-3α to the OH proton. This coupling, which we have sometimes observed (without comment) among a variety of other 3β-hydroxysterols in $CDCl_3$ solution, is routinely present in dimethylsulfoxide-d_6 spectra and is attributable to slow exchange of the hydroxyl proton. Similar couplings were also frequently observed for hydroxyl at other positions.

2.27. Stereochemical NMR assignments of the side chain methylene protons

Assignments for 25-norketone **30** were based on previously described conformational analyses of sterol side chains (Swaminathan et al., 1993; Wilson et al., 1994). The downfield C-22 proton (δ_H 1.342; dddd, 13.3, 11.4, 5.7, 2.9 Hz) was assigned as pro-*R* based on its small coupling (2.9 Hz) to H-20 as well as on chemical shift comparisons with other sterols described herein. Matching coupling constants of the downfield C-23 proton (δ_H 1.652; ddddd, 13.5, 11.2, 8.8, 6.4, 4.7 Hz) with those of H-22*R* indicated a 11.3 Hz mutual coupling. Thus, the two protons are *anti* in the predominant extended conformation, and the downfield C-23 proton is pro-*S*. These coupling assignments were confirmed in the double-quantum filtered COSY spectrum, which also showed active couplings from H-23*R* to the upfield and downfield C-24 protons of 8.6 and 6.2 Hz, respectively. Based on molecular modeling results showing the extended side chain conformation to be favored over the other C23–C24 rotamers by 0.7 kcal/mol, this establishes the upfield C-24 proton as pro-*R*. The similarity of the downfield shifts for H-23*R* and H-23*S* (0.30, 0.31 ppm) and for H-24*R* and H-24*S* (1.26, 1.28 ppm) relative to corresponding protons of cholesteryl acetate (Wilson et al., 1996) suggests that, in the overall distribution of side chain conformers, the C-25 carbonyl and methyl groups are each symmetrically disposed about the plane bisecting the side chain (in its extended conformation). The effect of the heptafluoro substitution in F_7-cholesterol also produced almost identical downfield shifts for diastereotopic methylene side-chain protons, which have been assigned previously (Swaminathan et al., 1993; Wilson et al., 1994). Chemical shift comparisons with the NMR data for F_7-cholesterol provided the corresponding assignments for the F_7 sterols described herein. Stereochemical assignments for the side-chain protons of 26-hydroxysterols will be described elsewhere.

2.28. NMR structure determination of Δ^4-3,7-dione **23d** and its Δ^5 isomer

Inspection of ^{1}H, ^{13}C, COSYDEC, HSQC, HMBC, and NOE difference spectra readily furnished complete ^{1}H and ^{13}C NMR assignments for the major component of the 3:1 mixture of 3,7-dione isomers except for the ene–dione system. Comparison of observed ^{1}H and ^{13}C NMR chemical shifts with those predicted for the Δ^4- and Δ^5-3,7-dione isomers (based on substituent increments for 7-keto and 26-hydroxy groups) suggested the major component to be the Δ^4 isomer: predicted for C-2 of Δ^4, Δ^5, δ_C 33.4, 37.1; observed for major, minor components, δ_C 33.6, 37.0; predicted for C-8, δ_C 49.1, 45.3; observed, δ_C 49.3, 45.3. An HMBC correlation of the non-conjugated carbonyl (δ_C 206.5) to H-8 (δ_H 2.53) provided a more definitive assignment of the major component as the Δ^4 isomer. Irradiation of the major and minor H-19 signals in NOE difference experiments led to stereochemical assignments for the allylic methylene protons (H-6 or H-4). The partial NMR data for the minor component (notably ^{1}H and ^{13}C data for protonated carbons in rings A and B) were in reasonable agreement with predicted values for the Δ^5 isomer and with ^{1}H NMR data reported for cholest-5-ene-3,7-dione (Schabdach et al., 1998).

2.29. Deuterium isotope effects on NMR chemical shifts

Relative to their undeuterated analogs, the 16,16-dideuterio sterols showed upfield shifts for C-15 (0.21 ppm), C-17 (0.15 ppm), C-20 (0.05 ppm), H-14α (0.003 ppm), H-15α (0.015 ppm), H-15β (0.015 ppm), H-17α (0.014 ppm), and H-18 (0.002 ppm). Similar upfield shifts were observed for **11**: C-15 (0.13 ppm), C-17 (0.08 ppm), C-20 (0.05 ppm), H-15α (0.008 ppm), H-15β (0.008 ppm), H-17α (0.004 ppm), and H-18 (0.002 ppm). Shieldings of other nuclei were essentially unchanged (≤ 0.002 ppm for ^{1}H and ≤ 0.02 ppm for ^{13}C). The 26,26,26,27,27,27-hexadeuteriosterols showed similar upfield shifts for C-24 (0.13 ppm), C-25 (0.49 ppm), and H-25, (0.038 ppm). The d_3-25-hydroxysterol preparation (**1f**, **1g**) showed upfield shifts for C-24 (0.06 ppm), C-25 (0.11 ppm), C-27 (0.06 ppm; diminished intensity), and H-27 (0.005 ppm)

3. Discussion

3.1. Chemical syntheses

We have prepared 43 oxygenated sterols with a diversity of functionality. Functional groups in rings A and B include Δ^5-3β,4β-dihydroxy (**3**), Δ^5-3β,7α-dihydroxy (**20**), Δ^5-3β,7β-dihydroxy (**21**), Δ^5-3β,19-dihydroxy (**29**), 3β,5α,6β-trihydroxy (**8**), 3β-hydroxy-5α,6α-epoxy (**5**), 3β-hydroxy-5β,6β-epoxy (**7**), Δ^5-3β-hydroxy-7-keto (**17**), and Δ^4-3-keto derivatives of 7,26-oxygenated sterols (**22–25**). Substituents on the parent C_8H_{17} side chain (**a**) include 25,26,26,26,27,27,27-heptafluoro (**b**), 26,26,26,27,27,27-hexadeuterio (**c**), (25*R*)-26-hydroxy (**d**), [16,16-$^2H_2$]-(25*R*)-26-hydroxy (**e**), and 25-hydroxy with deuteration at one of the terminal methyls (**g**, **h**). The deuterated and fluorinated sterols were prepared for use as internal standards to facilitate the quantitation of oxysterols by GC-MS. The fluorinated sterols are also of interest because of their anticipated blockage of oxidative metabolism at the side-chain terminus.

The parent oxygenated sterols (**a**) described herein and their 25,26,26,26,27,27,27-heptafluoro (**b**) and 26,26,26,27,27,27-hexadeuterio (**c**) derivatives were synthesized from cholesterol (**1a**), F_7-cholesterol (**1b**), d_6-cholesterol (**1c**), or their acetate derivatives **2a–c** by modifications of previously described approaches. As shown in Fig. 1, the 4β-hydroxysterols **3a–c** were prepared in 34–41% yield by oxidation of the Δ^5-steryl acetates **2a–c** with selenium dioxide (Rosenheim and Starling, 1937) followed by saponification of the acetate. Oxidation of the Δ^5-steryl acetates **2a–c** with *m*-chloroperbenzoic acid gave predominantly the α-epoxide acetates **4a–c**, which were purified (Kudo et al., 1989) by alumina–$AgNO_3$ chromatography prior to saponification to the free sterols **5a–c**. The epoxidation reactions proceeded in 56–77% yield and the saponifications in 88–91% yield. The usual preference for α-epoxidation (owing to steric effects of the C-10 methyl group) can be reversed by blocking the α-face of the Δ^5 bond with a transition metal ion under specific reaction conditions (Parish and Li, 1996 and ref. therein). Thus, potassium permanganate oxidation of **2a–c** in the presence of copper sulfate (Syamala et al., 1992) followed by MPLC purification on alumina–$AgNO_3$ afforded the β-epoxide acetates **6a–c** in 55–85% yield, and saponification gave the free sterols **7a–c** in 86–94% yield. This route represents a useful alternative to the classical synthesis of β-epoxide **7a** via the bromohydrin **26a** (Fried and Sabo, 1957), especially for small-scale syntheses. Triols **8a–c** were prepared in 69–78% yield by oxidation of the Δ^5 free sterols **1a–c** with hydrogen peroxide in formic acid, followed by saponification of any formate esters formed during the reaction (Fieser and Rajugopalan, 1949).

As shown in Fig. 2, [16,16-$^2H_2$]-(25*R*)-cholest-5-ene-3β,26-diol diacetate (**2e**) was prepared in 53% yield from (25*R*)-bis(3β,26-*tert*-butyldimethylsilyloxy)cholest-5-en-16-one (**9**), which has been synthesized from diosgenin in 37% yield (Kim et al., 1989). The synthesis of **2e** from diosgenin requires three chromatographic separations but can readily be carried out on a gram scale. The free sterol **1e** was obtained in 89% yield by saponification of acetate **2e**. Deuterium was introduced specifically at C-16 by two reductions with $LiAlD_4$, first on 16-ketosterol **9**, and then on mesylate **12**. Use of $LiAlD_4$ of $\geq$ 99% isotopic purity led to high levels of deuterium incorporation at C-16. Mass spectral analysis showed $\sim$99% d_2 for **2e**, and ^1H and ^{13}C NMR spectra indicated the virtual absence of 16-protio material. ^2H NMR demonstrated the absence of deuterium at other positions. The $LiAlD_4$ reductions produced minor byproducts **14** (characterized as diacetate **15**) and **11**, which were separated from the main products and identified by NMR. The present synthesis of (25*R*)-26-hydroxycholesterol, with a high incorporation of the isotopic label at a single carbon atom, has clear advantages over previous syntheses of the deuterated sterol (Shoda et al., 1993b; Ni et al., 1994; D'Ambra et al., 1997; and Ref. therein). Moreover, the label at C-16 is at a relatively inert position with respect to known sterol metabolism and to mass spectral fragmentation.

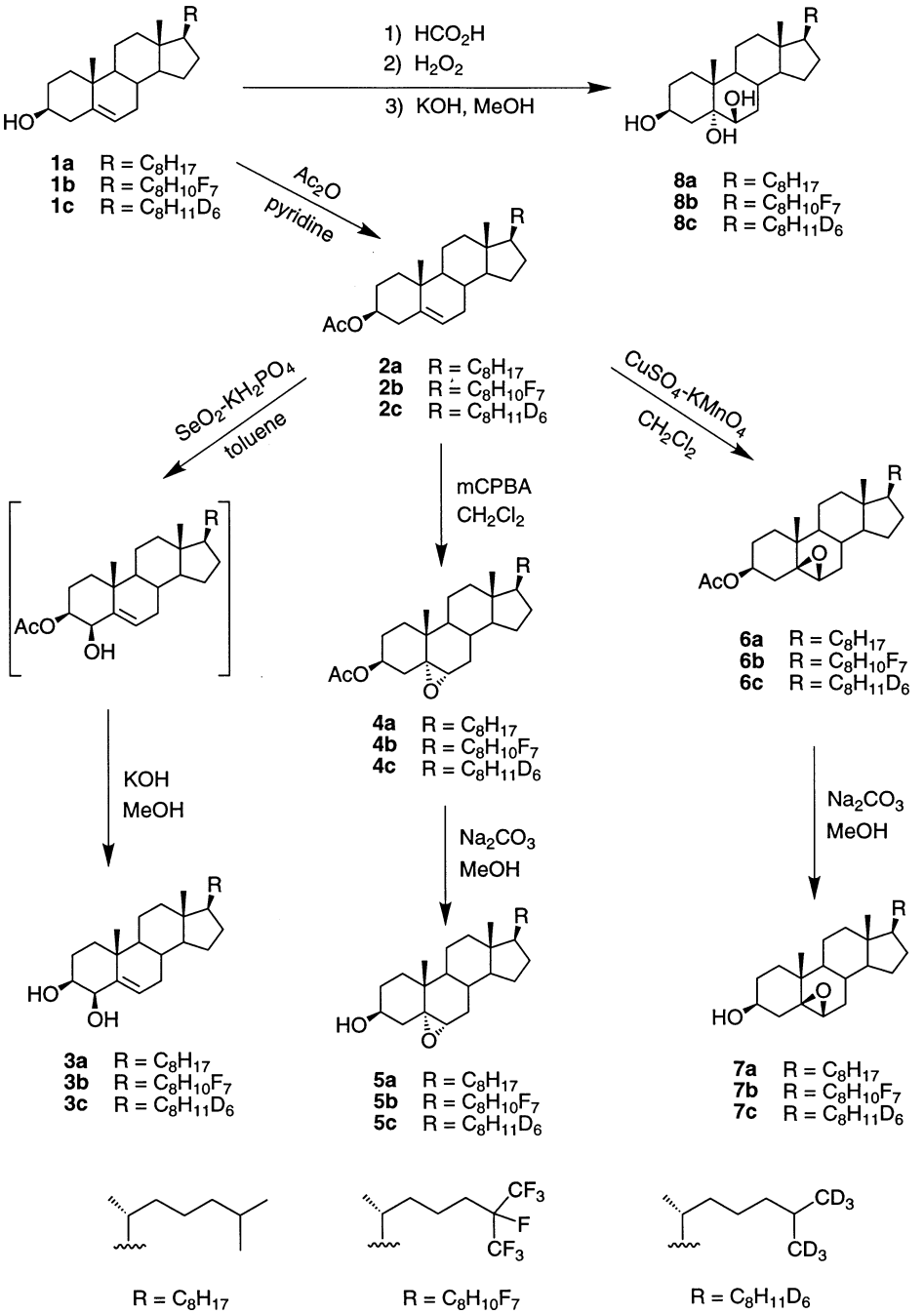


Fig. 1. Synthesis of cholest-5-ene-3β,4β-diol (**3a**), 5,6α-epoxy-5α-cholestan-3β-ol (**5a**), 5,6β-epoxy-5β-cholestan-3β-ol (**7a**), 5α-cholestane-3β,5,6β-triol (**8a**), and their 25,26,26,26,27,27,27-heptafluoro and 26,26,26,27,27,27-hexadeuterio derivatives.

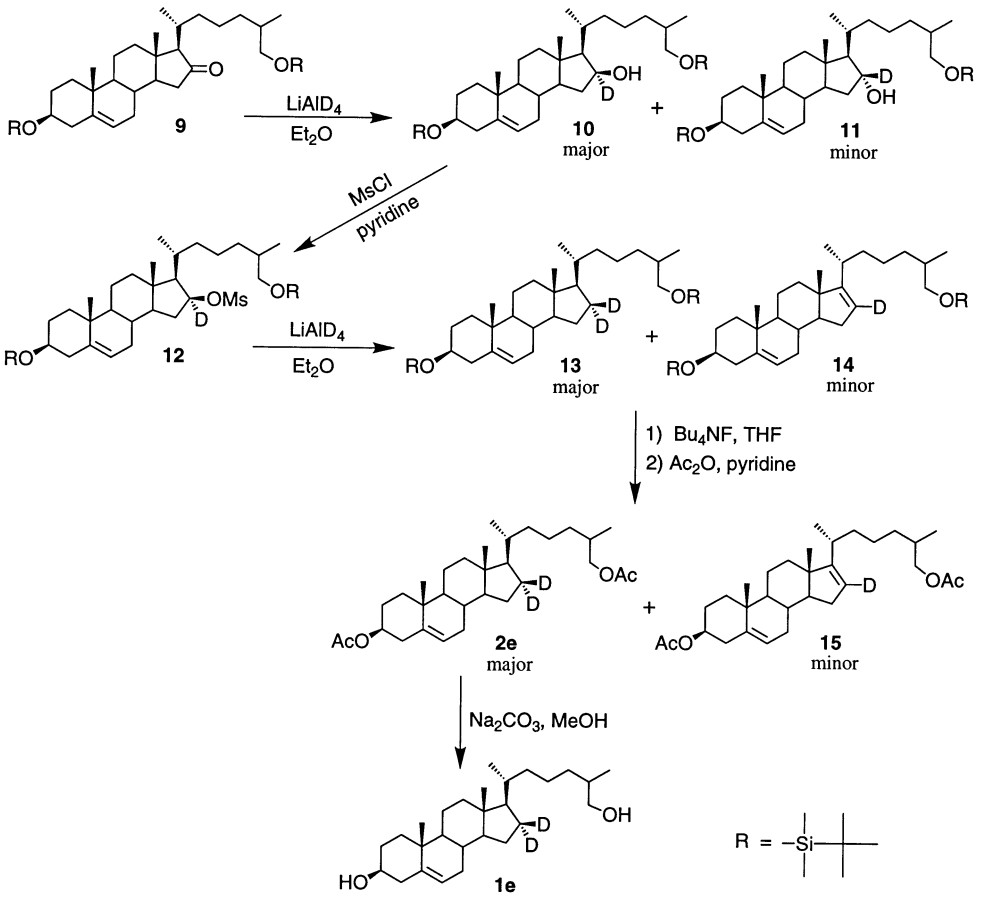


Fig. 2. Synthesis of deuterium-labeled (25*R*)-26-hydroxycholesterol (**1e**) and its diacetate derivative (**2e**).

The Δ^5-7-ketosterols **17a–c** were prepared as shown in Fig. 3 from the Δ^5-steryl acetates **2a–c** in 55–61% overall yield by oxidation with pyridinium chlorochromate in refluxing benzene, followed by saponification (Parish et al., 1986; Parish and Wei, 1987). Lithium aluminum hydride reduction (Cheng et al., 1977) of the Δ^5-7-keto acetates **16a–c** gave a 3:1 mixture of the 7β- and 7α-hydroxysterols, which could be separated as the diacetates (**18a–c**, **19a–c**) by MPLC on alumina–$AgNO_3$ as reported previously by Kudo et al. (1989). Saponification of the diacetates gave the 7α-hydroxysterols **20a–c** (16–19% yield from **16a–c**) and the 7β-hydroxysterols **21a–c** (53–63% yield from **16a–c**). The 7-keto, 7α-hydroxy, and 7β-hydroxy sterols were also prepared similarly as their (25*R*)-26-hydroxy derivatives (**17d**, **20d**, and **21d**) and [16,16-$^2H_2$]-(25*R*)-26-hydroxy derivatives (**17e**, **20e**, and **21e**) from (25*R*)-26-hydroxycholesterol diacetates **2d** and **2e**. The 7α-hydroxy sterols **20d** and **20e** were obtained by reduction of the 7-ketosterols with L-Selectride, which gives almost exclusively the 7α isomer (Kumar et al., 1987).

As shown in Fig. 4, (25*R*)-cholest-5-ene-3β,26-diol (**1d**), its 7α- and 7β-hydroxy derivatives (**20d**, **21d**), and their 16,16-dideuterio derivatives (**1e**, **20e**, **21e**) were regioselectively oxidized/isomerized to the Δ^4-3-ketosteroids (**22d**, **22e**, **24d**, **24e**, **25d**, **25e**) on a 20–60 mg scale with cholesterol oxidase. This enzyme can be regarded a useful reagent for selectively oxidizing the 3β-hydroxyl of oxygenated sterols. Reactions on a 10–100 mg scale require attention to the limited solubility of sterols in aqueous medium and the limited stabil-

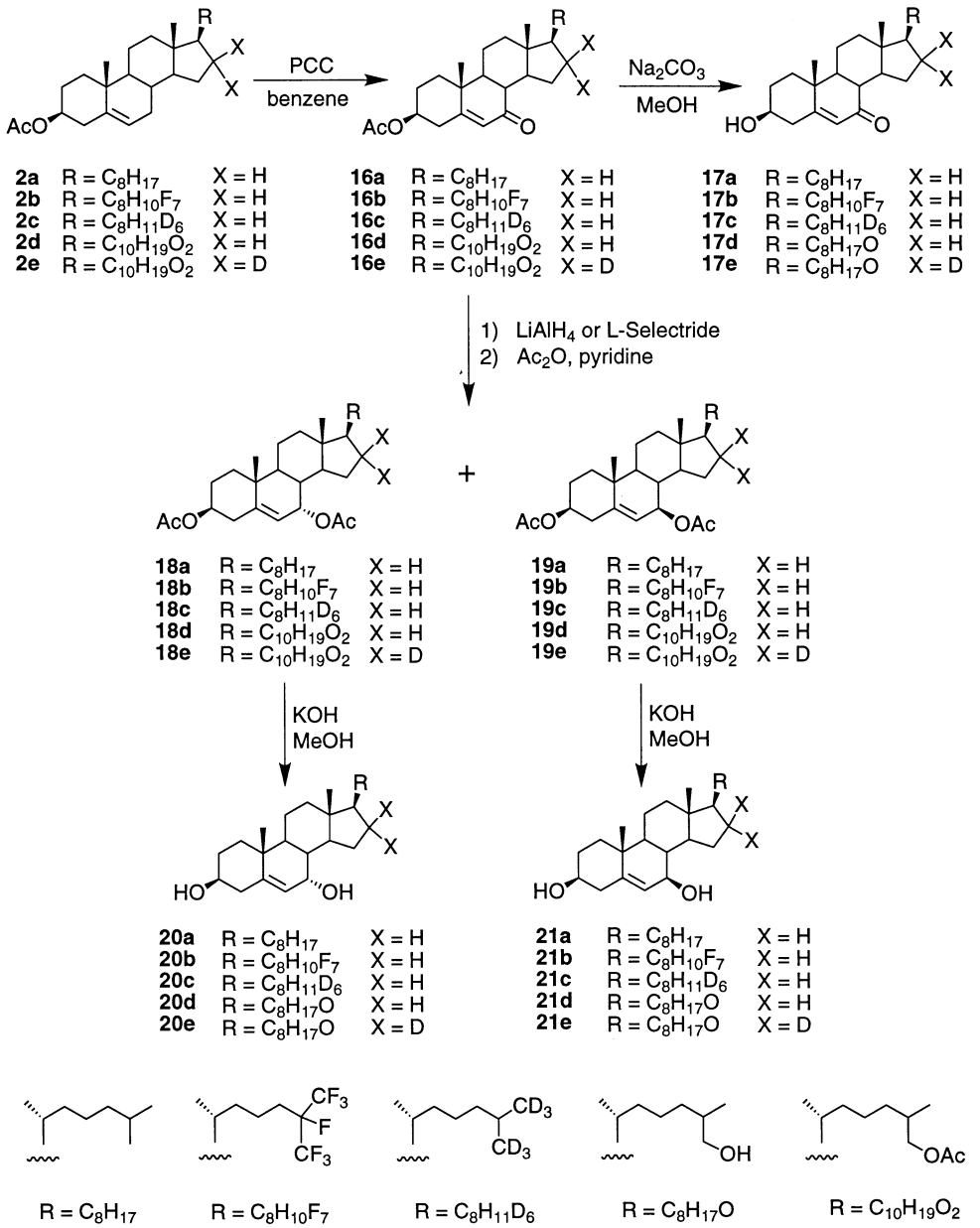
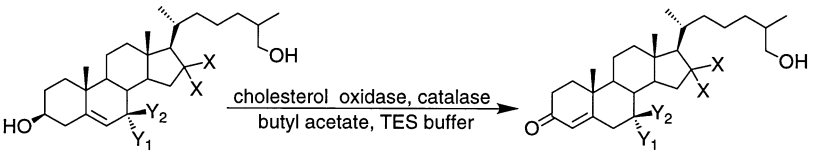


Fig. 3. Synthesis of 3β-hydroxycholest-5-en-7-one (**17a**), cholest-5-ene-3β,7α-diol (**20a**), its 7β-epimer (**21a**), and their derivatives containing 25,26,26,26,27,27,27-heptafluoro, 26,26,26,27,27,27-hexadeuterio, (25*R*)-26-hydroxy, and [16,16-$^2H_2$]-(25*R*)-26-hydroxy substitution.

ity of cholesterol oxidase in organic solvents. Effective solutions to these problems include the use of isopropyl alcohol (Schroepfer et al., 1977) or detergents to solubilize the sterol substrates, adsorption of the sterol substrate onto silica gel (Lee and Biellmann, 1988) or hydroxypropyl-β-cyclodextrin (Alexander and Fisher, 1995; Labaree et al., 1997), and use of a two-phase solvent system (Lee and Biellmann, 1988). By stirring a concentrated sterol solution in butyl acetate with



1d	Y_1 = H	Y_2 = H	X = H	**22d**	Y_1 = H	Y_2 = H	X = H
1e	Y_1 = H	Y_2 = H	X = D	**22e**	Y_1 = H	Y_2 = H	X = D
17d	Y_1 = O	Y_2 = O	X = H	**23d**	Y_1 = O	Y_2 = O	X = H
20d	Y_1 = OH	Y_2 = H	X = H	**24d**	Y_1 = OH	Y_2 = H	X = H
20e	Y_1 = OH	Y_2 = H	X = D	**24e**	Y_1 = OH	Y_2 = H	X = D
21d	Y_1 = H	Y_2 = OH	X = H	**25d**	Y_1 = H	Y_2 = OH	X = H
21e	Y_1 = H	Y_2 = OH	X = D	**25e**	Y_1 = H	Y_2 = OH	X = D

Fig. 4. Regioselective oxidation of (25*R*)-26-hydroxycholesterol (**1d**), its 7-oxygenated derivatives, and their 16,16-dideuterio derivatives by cholesterol oxidase.

a small volume of aqueous buffer/enzyme, we obtained, with one exception, virtually complete oxidation within 30 h to the Δ^4-3-ketosteroids, which were isolated in high yield and high purity. No byproducts were observed, although the 7-hydroxy-Δ^4-3-ketosteroids (**24d**, **24e**, **25d**, **25e**) readily underwent elimination of the allylic hydroxyl[6], as has been noted previously for related steroids (Brooks et al., 1983; Labaree et al., 1997). Unlike the other sterols, (25*R*)-3β,26-dihydroxycholest-5-en-7-one (**17d**) and its 16,16-dideuterio derivative **17e** were oxidized very poorly. Workup of the reactions gave mainly the starting 3β-hydroxysterols, but 3:1 mixtures of the Δ^4- and Δ^5-3,7-diones were isolated in low yield. This observation is in accord with reports that 7-ketocholesterol is at best a poor substrate for cholesterol oxidase (Ögren et al., 1980; Slotte, 1992).

Previous syntheses of the Δ^4-3-ketosteroids **22d**, **24d**, and **25d** with cholesterol oxidase were done on a 0.5–1 mg scale, and product characterization was limited to GC-MS of the TMS ethers (Shoda et al., 1993b). Described herein for these compounds and their 16,16-dideuterio derivatives (**22e**, **24e**, and **25e**) are melting points and definitive spectroscopic characterization. The 3,7,26-trioxygenated sterols are of interest not only for their role in cholesterol metabolism (Axelson and Sjövall, 1990) but also for their possible regulatory effects (Axelson et al., 1995; Axelson and Larsson, 1996), their cytostatic properties (Axelson and Larsson, 1997), and their close structural relationship to pavoninins, sterol monoglycosides that are the active components in ichthyotoxic secretions produced by certain species of fish (Tachibana et al., 1984; Ohnishi and Tachibana, 1997). On a small scale, our regioselective enzymatic oxidation of the 3β,7α,26-triol **20d** furnishes an attractive alternative to a recently described route to the aglycone of pavoninin-2 (**24d**), which was prepared via selective protection of (25*R*)-26-hydroxycholesterol as its 26-TBDMS ether (Kim et al., 1997).

19-Hydroxycholesterol and its F_7 and d_6 derivatives (**29a–c**) were prepared by known methodology (Kalvoda et al., 1963) shown in Fig. 5. Treatment of the Δ^5-steryl acetates **2a–c** with *N*-bromoacetamide and catalytic amounts of perchloric acid gave 2:1 mixtures of the 5α-bromo-6β-hydroxy and 5α-hydroxy-6β-bromo isomers. Oxidation of these crude[7] bromohydrin

[6] For example, ^{1}H NMR spectra of a sample of **24d** showed 99% purity in $CDCl_3$ filtered through basic alumina but a 7:1 ratio of **24d** and (25*R*)-26-hydroxycholesta-4,6-dien-3-one in unfiltered $CDCl_3$. ^{1}H NMR of the $\Delta^{4,6}$-dienone (50 mM): δ_H 6.146 (dd, 9.8, 2.0 Hz), 6.096 (dd, 9.8, 2.7 Hz), 5.668 (t, 0.8 Hz), 3.500 (dd, 10.4, 5.9 Hz), 3.425 (dd, 10.5, 6.5 Hz), 2.573 (ddd, 17.8, 14.5, 5.4 Hz), 1.111 (d, 0.7 Hz, 3H), 0.921 (d, 6.6 Hz, 3H), 0.916 (d, 6.8 Hz, 3H), 0.755 (d, 0.5 Hz, 3H).

[7] The desired bromohydrin **26a** could be isolated in low yield by recrystallization, and the cyclic ether could be separated from the α-epoxide byproduct by repetitive MPLC purification on silica gel. However, chromatographic purification of the cyclic ethers **27a** and **27c** introduced a few percent of olefinic contaminants. Delaying purification until the stage of the 19-hydroxysteryl acetate resulted in high product purity and better overall yields.

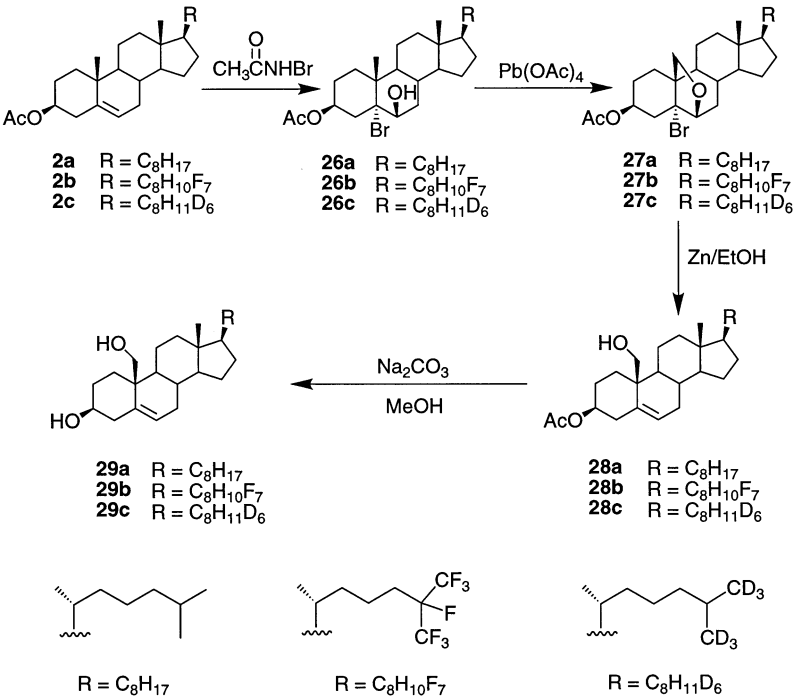


Fig. 5. Synthesis of cholest-5-ene-3β,19-diol (**29a**) and its 25,26,26,26,27,27,27-heptafluoro and 26,26,26,27,27,27-hexadeuterio derivatives.

mixtures with lead tetraacetate in the presence of calcium carbonate converted the major bromohydrin isomer to the 6β,19 cyclic ethers **27a–c** and the minor bromohydrin to α-epoxide acetates **4a–c**. Reductive cleavage with zinc dust afforded the 19-hydroxysteryl monoacetates **28a–c**, which were separated from the α-epoxide byproduct by MPLC and then solvolyzed to the free sterols **29a–c** (23–35% yield from **2a–c**). d_3-25-Hydroxycholesterol was prepared in 95% yield by Grignard addition of deuterated methyl magnesium iodide to 25-norketone **30** (Fig. 6). ^1H and ^{13}C NMR signal intensities for the terminal methyl groups[4] indicated a 1:1 mixture of 25*R* and 25*S* epimers **1g** and **1h**.

It is important to note that deuterium-labeled analogs of a number of oxysterols have been prepared previously by others. However, in each case, very little or no characterization of the sterols was presented. A number of investigators have employed the Clemmensen reduction of kryptogenin in deuterated medium to prepare deuterium-labeled (25*R*)-cholest-5-ene-3β,26-diol (Javitt et al., 1981, 1982; Breuer and Björkhem, 1990; Shoda et al., 1993b; Dzeletovic et al., 1995). However, little characterization of the products has been presented, and the published MS data presented for one of the products (Breuer and Björkhem, 1990) contain a number of ions not found in the authentic standard. The lack of characterization is important since reinvestigations of the Clemmensen reduction of kryptogenin (Kluge et al., 1985) and diosgenin (Ni et al., 1993) have demonstrated the complexity of this procedure and of previously undescribed byproducts. The preparation of [26,26,26-$^2H_3$]cholest-5-ene-3β,25-diol has also been described previously (Breuer and Björkhem, 1990; Dzeletovic et al., 1995); however, little or no characterization of the product was presented. The preparation of [25,26,26,26,27,27,27-$^2H_7$]3β-hydroxycholest-5-en-7-one has been described previously (Björkhem, 1986; Breuer and Björkhem, 1990), with no characterization except for partial MS data of its TMS

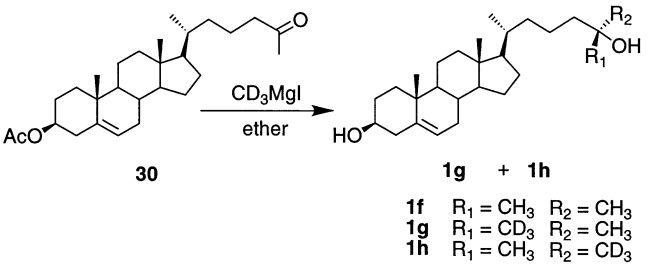


Fig. 6. Synthesis of deuterium-labeled 25-hydroxycholesterol (**1g**, **1h**) as a mixture of C-25 epimers.

derivative (Breuer and Björkhem, 1990). Breuer and Björkhem (1990) described the preparation of [25,26,26,26,27,27,27-$^2H_7$]5,6-epoxycholestan-3β-ol as a 9:1 mixture of its 5α,6α- and 5β,6β-isomers, [25,26,26,26,27,27,27-$^2H_7$]cholestane-3β,5α,6β-triol, and [26,26,26-$^2H_3$]cholest-5-ene-3β,25-diol; however, no details of the synthetic work or characterization of the products were presented other than partial MS data on the TMS derivative. Dzeletovic et al. (1995) reported the syntheses of [26,26,26,27,27,27-$^2H_6$]3β-hydroxycholest-5-en-7-one and the corresponding d_6 analogs of cholest-5-ene-3β,7-diol (as an unresolved mixture of the 7α and 7β isomers), 5α,6α-epoxycholestan-3β-ol (containing 5% of the 5β,6β-epoxide), and cholestane-3β,5α,6β-triol. Björkhem and Kallner (1976) described the preparation of [3β,4β,7β-$^2H_3$]cholest-5-ene-3β,7α-diol; however, no characterization of the product was presented. The preparation of [26,26,26,27,27,27-$^2H_6$]cholest-5-ene-3β,4β-diol was made by Breuer (1995); however, no characterization of the product was presented other than a partial MS of its TMS derivative. Krut et al. (1997) described the conversion of [26,26,26,27,27,27-$^2H_6$]cholesterol to a mixture of the corresponding d_6 analogs of 7-ketocholesterol, 7α-hydroxycholesterol, and 7β-hydroxycholesterol. The mixture was apparently not resolved and no characterization data were presented.

3.2. Purification, characterization and spectral analysis

The oxysterols described herein were obtained in high purity (generally 98–99%)[8]. Purities were estimated from 500 MHz ^1H NMR spectra, methodology that we have found to be quite sensitive and frequently more reliable than chromatographic methods for determining the purity of sterol samples. NMR spectra of 1 mg samples typically revealed sterol impurities with a detection limit of 0.1%. Sample purities were also monitored by TLC.

Alumina–$AgNO_3$ chromatography was essential in the purification of many of the oxysterols. For example, crude preparations of epoxides **4a–c** and **6a–c** were invariably mixtures containing both α and β isomers, for which we observed identical mobilities by TLC as either the free sterols or the acetates. Kudo et al. (1989) resolved the parent diacetate isomers (**4a**, **6a**) by MPLC and HPLC on silica gel but obtained far better separations on alumina–$AgNO_3$. Our purification of six crude epoxide reaction mixtures (**4a–c**, **6a–c**) as the diacetates on alumina–$AgNO_3$ furnished the individual isomers cleanly with good recovery of material. Separation of the 7α- and 7β-hydroxysterols (**20a–e** and **21a–e**) presented a similar problem. With our TLC solvent systems, we observed little or no resolution of the 3β,7-diols and their diacetates on silica gel. Others have also considered the parent free sterols (Alexander and Fisher, 1995) and diacetates (Kudo et al., 1989) to be chromatographically inseparable on silica gel, although separations of the free sterols

[8] The slightly lower purities of several of the F_7-oxysterols were traced to the use of a sample of F_7-cholesterol containing 1–2% (23*E*)-25,26,26,26,27,27,27-heptafluorocholesta-5,23-dien-3β-ol.

have been reported (Johnson and Lack, 1976; Kudo et al., 1989). Following the work of Kudo et al. (1989), we have found alumina–$AgNO_3$ MPLC separation of the diacetates (**18a–e**, **19a–e**) to be highly effective. The striking resolution of the 3β,7-diacetate epimers and of the 5,6-epoxy isomers demonstrates the value of alumina–$AgNO_3$ for chromatographic separations that are difficult or impossible on silica gel. Argentation chromatography has traditionally been limited mainly to separation of double bond isomers, but these and other (Ruan et al., 1999) recently reported examples indicate that this methodology may have more general application.

The F_7- and d_6-oxysterols were characterized by melting point, TLC, IR, MS, high-resolution MS, and ^1H and ^{13}C NMR. The final F_7-oxysterol products were also characterized by ^{19}F NMR, and ^2H NMR data were obtained for representative d_6-oxysterols. The spectral data were compatible with the structures presented, and the effects of the F_7 substitution were consistent with those observed for other F_7 sterols (Swaminathan et al., 1993, 1995; Siddiqui et al., 1997). Complete ^1H and ^{13}C NMR signal assignments are presented in Tables 1–4 for the F_7-oxysterols described herein. These assignments were established primarily from HSQC and COSYDEC spectra in conjunction with 1D spectra, as described previously (Wilson et al., 1996). Stereochemical assignments were established for the side-chain protons of 25-norketone **30** (from ^1H NMR coupling constants of resolved resonances) and for the diastereotopic C-19 protons of the 6β,19-cyclic ether **27a** and 19-hydroxysterols **28a–c** and **29a–c** (from NOE difference experiments). The NOE results for the 19-hydroxysterols were consistent with molecular mechanics calculations indicating that the 19-hydroxyl is positioned mainly over ring B. In each case, the C-19 proton over ring A (pro-*R*) was more deshielded its geminal partner. ^1H and ^{13}C NMR spectral data were measured under conditions of reproducibly high precision (generally ± 0.001 ppm for ^1H and ± 0.03 ppm for ^{13}C) by careful attention to temperature, sample concentration, effects of strong coupling, and avoidance of solvent impurities. The high precision of the chemical shift measurements together with the high spectral resolution and 2D NMR results permitted the identification of many minor reaction products. Knowledge of the nature and amounts of these byproducts was useful in optimizing reaction conditions and in formulating strategies for product purification.

Electron-impact mass spectra of the oxysterols described herein were obtained under uniform conditions by direct probe on a double-focusing sector instrument. Interpretation of the spectra was facilitated by high-resolution data for most of the fragment ions and by the availability of oxysterols with deuterium or fluorine labeling at the side-chain terminus. In addition to the usual fragment ions corresponding to loss of CH_3, H_2O, side chain, and combinations thereof, the oxysterols produced in lower abundance ions resulting from cleavage in rings B and C, as illustrated in Fig. 7 (panels A and B). The $C_8H_{10}F_7$ side-chain ion (m/z 239) was observed in moderate abundance for many F_7-oxysterols, but the corresponding ion for sterols with a C_8H_{17} or $C_8H_{11}D_6$ side chain had negligible abundance.

Some oxysterols exhibited distinctive fragmentation patterns. For example, the 19-hydroxy-Δ^5 sterols **29a–c** showed M − 30 and M − 31 ions, which were attributable to loss of formaldehyde via a McLafferty-type rearrangement or to loss of the CH_2OH radical (Fig. 7C). Facile transfer of hydrogen from the 19-hydroxyl to C-6 is compatible with the predominant 19-hydroxyl conformation observed in NOE difference experiments and predicted from molecular mechanics calculations[9]. Loss of formaldehyde together with H_2O represented the base peak in each spectrum of free sterols **29a–c**, and loss of the CH_2OH radical with

[9] At the high ionizer temperatures (ca. 200°C), population differences among conformers are somewhat equalized, but the low-energy conformer still predominates. For example, if the three conformers have relative energies of 0.0: 0.3: 1.5 kcal/mol (as calculated by molecular mechanics), the population distribution is 60:36:5 at 25°C and 52:38:11 at 200°C. However, it should be acknowledged that the hydroxyl hydrogen is anti to C-10 (and remote from C-6) in the predominant conformation and that calculations were done on the neutral sterol rather than the radical cation.

CH_3COOH corresponded to the base peak of acetates **28a–c**. Compared with the free sterols, acetates **28a–c** showed lower abundances for loss of CH_2O but higher abundances for loss of CH_2OH radical and $CH_2O + H_2$ (which may involve aromatization of ring A after loss of CH_3COOH). These types of fragmentation were not observed for any other oxysterols. d_3-25-Hydroxycholesterol (**1g**, **1h**) showed fragmentation initiated at the 25-oxygen and leading to M − SC–2 ions as well as the usual M − SC ions. Fragmentation in rings A and B led to minor ions at M − 85 and M − 111 and, in conjunction with thermal elimination of the 25-hydroxyl, more abundant ions at *m/z* 276 and 302. Similar fragmentations of cholesterol (Wyllie et al., 1977) and of sterols with unsaturated side chains (Wyllie and Djerassi, 1968; Massey and Djerassi, 1979) have been studied in detail.

The availability of MS data for the parent oxysterols as well as their F_7 and d_6 derivatives permitted an unusually reliable assessment of the distinguishing spectral characteristics of the oxysterols. After consideration of the variability in the abundance of ions arising partially from thermal elimination of water, mass spectra of the 7α-hydroxy and 7β-hydroxysterols **20a–c** and **21a–c** were essentially identical, as were spectra of their diacetates **18a–c** and **19a–c**. Spectra of the α- and β-epoxide isomers **4a–c**, **5a–c**, **6a–c**, and **7a–c** also initially appeared identical within the expected variabilities of ion abundances. However, presentation of the epoxide data in tabular form (Table 5) revealed several ions that might be used to assign α or β stereochemistry. The free sterols may be distinguished by the lower abundances for M − CH_3, M − H_2O–CO, and M − H_2O–CHO in the α isomer, and the acetate isomers may be differentiated by similar abundance patterns for the latter two ions. Because the cited differences in ion abundances are modest and because ion abundances vary depending on the type of instrument and the tuning of the ion optics, assignment by MS alone of the stereochemistry of an epoxide unknown would likely require comparison with authentic standards.

Mass spectra of the starting d_6-cholesterol and the d_6-oxysterols derived therefrom indicated a high level of isotope incorporation.[10] ^1H, ^2H, and ^{13}C NMR spectra demonstrated the presence of deuterium only at the terminal methyl positions.

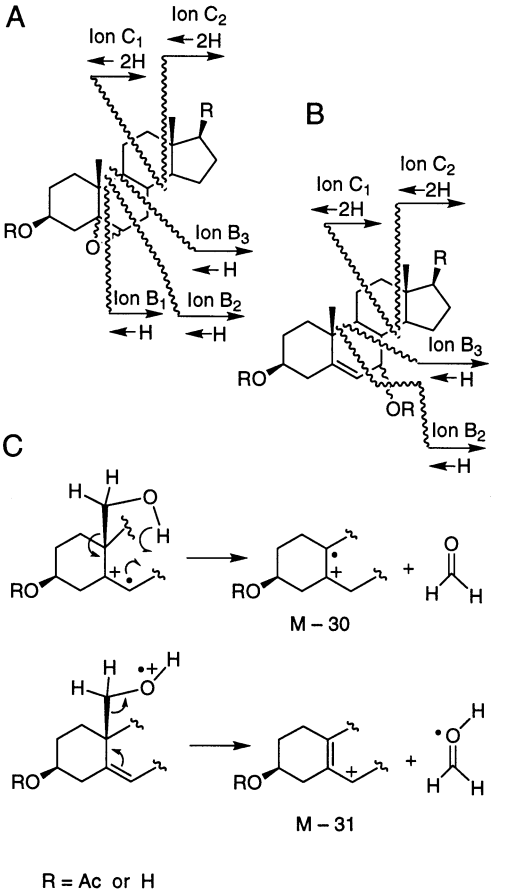


Fig. 7. Sites of electron-impact mass spectral fragmentation: panel A, cleavage of 5,6-epoxides in rings B and C; panel B, cleavage of 7-hydroxy-Δ^5 sterols in rings B and C; panel C, suggested mechanism for loss of formaldehyde by a McLafferty-type rearrangement and for loss of the CH_2=OH radical. The M − 31 ion can arise from initial ejection of an oxygen electron followed by electron migration (as shown) or by initial ejection of an olefinic electron followed by a simple redistribution of bonding electrons.

[10] Ion abundances (1–3%) for d_3, d_4, and d_5 species of the d_6-sterols were comparable with the variable abundances (1–5%) observed for M − 1 and M − 2 of undeuterated sterols as a consequence of catalytic or thermal dehydrogenation and other reactions. Additional ambiguity in estimating levels of deuterium incorporation arose for acetates and other species lacking an intense molecular ion.

The mass spectrum of d_3-25-hydroxycholesterol, prepared as a mixture of C-25 epimers (**1g** and **1h**) labeled at C-26, also showed a high level of deuterium incorporation (ca. 98%), and NMR spectra indicated that deuterium was located only on the terminal methyl groups. As noted above, MS and NMR showed the 16,16-dideuteriosterols to have high isotopic incorporation ($\sim 99\%$ d_2), with the isotopic label exclusively at C-16.

The deuterated and fluorinated oxysterols described herein should prove valuable as internal standards in GC-MS studies of the levels of oxysterol in blood and tissues. Moreover, the fluorine substitution in the F_7-oxysterols should be useful for blockage of potential oxysterol metabolism initiated by oxidation at C-25, C-26, or C-27. The syntheses of F_7-substituted 7-ketocholesterol (Carroll et al., 1998) and of 15-ketosterols (Swaminathan et al., 1993, 1995; Siddiqui et al., 1997) for this purpose have been described previously.

Acknowledgements

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