Inhibitors of sterol synthesis. Spectral characterization of derivatives of 5α-cholest-8(14)-en-3β-ol-15-one

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Abstract Described herein are the chemical syntheses of a number of deuterated derivatives of 5α-cholest-8(14)-en-3β-ol-15-one. These include the [2,2,3α,4,4,7,7,9α,16,16-$^2H_{10}$]-, [7α,9α,16,16-$^2H_4$]-, [7,7,9α,16,16-$^2H_5$]-, and [2,2,3α,4,4-$^2H_5$]-analogs of the $\Delta^{8(14)}$-15-ketosterol. Also included are the syntheses of the 3β-acetate derivatives of the latter three deuterated analogs and of 5α-cholest-8(14)-en-3β-ol-15-one, and 5α-cholest-8(14)-en-3α-ol-15-one. Low resolution mass spectral data on these compounds and on 5α-cholest-8(14)-en-15-one, 5α-cholest-8(14)-en-3β-ol-15-one, 5α-cholest-8(14)-en-3α-ol-15- one, 3β-benzoyloxy-5α-cholest-8(14)-en-15-one, and the trimethylsilyl ethers of the free sterols have been presented. The results of these studies, supplemented with high resolution mass spectral data on five of these compounds, have been used to evaluate the electron impact mass spectral fragmentation of the $\Delta^{8(14)}$-15-ketosterols and their derivatives. Also presented herein are the results of ^1H, ^2H, and ^{13}C nuclear magnetic resonance studies of 5α-cholest-8(14)-en-3β-ol-15-one and its derivatives.—**St. Pyrek, J., W. K. Wilson, and G. J. Schroepfer, Jr.** Inhibitors of sterol synthesis. Spectral characterization of derivatives of 5α-cholest-8(14)-en-3β-ol-15-one. *J. Lipid Res.* 1987. **28:** 1296–1307.

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5α-Cholest-8(14)-en-3β-ol-15-one **2** **(Fig. 1)** is a potent inhibitor of sterol biosynthesis in cultured mammalian cells and lowers the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase in these cells (1–3). In addition, the 15-ketosterol **2** has been shown to have marked hypocholesterolemic activity upon oral administration to rats (4, 5), mice (4), baboons (6), and rhesus monkeys (7). Moreover, this inhibitor of sterol biosynthesis also has the unique feature of itself serving as an efficient precursor of cholesterol. The enzymatic conversion of the 15-ketosterol **2** to cholesterol has been demonstrated in rat liver homogenate preparations (8) and in intact rats (9).

In the present study we have directed our efforts towards the preparation and characterization of a number of deuterated derivatives of 5α-cholest-8(14)-en-3β-ol-15-one **2**. These samples were required for use in: *a*), electron impact mass spectral fragmentation studies of the 15-ketosterol and its derivatives; *b*), quantitative determinations of **2** by mass fragmentography; *c*), ^1H and ^{13}C nuclear magnetic resonance (NMR) studies of the 15-ketosterol and its derivatives; and *d*), studies of the metabolism of the 15-ketosterol **2**.

EXPERIMENTAL

General

Melting points were measured with a Thomas-Hoover melting point apparatus using evacuated capillaries. Ultraviolet (UV) spectra were recorded on an IBM 9430 spectrophotometer (Danbury, CT) using hexane as the solvent. Thin-layer chromatography (TLC) was performed using either precoated 0.2-mm high performance TLC plates (silica gel 60; EM Science (Merck), Cherry Hill, NJ) or precoated 0.25-mm silica gel G plates (Analtech, Inc., Newark, DE). Substances were visualized by spraying with 5% ammonium molybdate in 10% sulfuric acid. All chemicals were purchased from Aldrich (Milwaukee, WI) or Mallinckrodt (Los Angeles, CA).

Nuclear magnetic resonance

^1H and ^{13}C NMR spectra were measured on an IBM AF300 spectrometer (Danbury, CT) in $CDCl_3$ solution in 5-mm tubes. Standard IBM software was used for data processing. ^1H NMR spectra were recorded at 300.1

Abbreviations: UV, ultraviolet; NMR, nuclear magnetic resonance; DEPT, distortionless enhancement by polarization transfer; HETCOR, ^1H-^{13}C heteronuclear correlation; FID, free induction decay; LIS, lanthanide-induced shifts; TLC, thin-layer chromatography; MS, mass spectra; GLC, gas–liquid chromatography; EI, electron impact; CI, chemical ionization; TMS, trimethylsilyl; BSTFA, *bis*-trimethylsilyl trifluoroacetamide; HRMS, high resolution mass spectra; SC, the alkyl side chain (C_8H_{17}) of the sterol.

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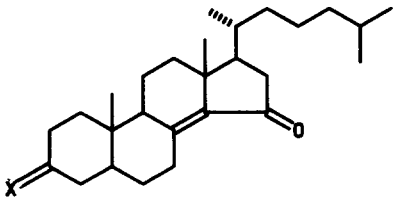

1	X:	H_2
2		αH, βOH
3		αH, βOAc
4		αH, βOBz
5		αH, βOTMS
6		βH, αOH
7		βH, αOAc
8		βH, αOTMS
9		O

Fig. 1. Structures of 5α-cholest-8(14)-en-15-one (**1**) and its derivatives.

MHz with a 5000 Hz spectral width, a 3.28 sec acquisition time, 40° pulses, 32k data points (digital resolution 0.31 Hz), and no line broadening. ^{13}C NMR spectra were recorded at 75.5 MHz with composite pulse decoupling using a 20,000 Hz spectral width, a 0.82 sec acquisition time, a 0.1 sec recycle delay, 34° pulses, 32k data points (digital resolution 1.2 Hz), and a 2 Hz line broadening. Proton spectra were referenced to tetramethylsilane at 0.0 ppm, and carbon spectra were referenced to $CDCl_3$ at 77.0 ppm. Carbon multiplicities were determined from 90° and 135° DEPT (distortionless enhancement by polarization transfer) spectra (10), which were measured under normal carbon conditions but with a recycle delay of 1 sec. Spectra showing only quaternary carbons were obtained using a special DEPT sequence (11) with a recycle delay of 3 sec. HETCOR (^{13}C-^{1}H shift-correlated two-dimensional NMR) spectra were obtained using a standard pulse sequence with decoupling in the F_1 (proton) dimension (12, 13). The spectral widths were 600 Hz (2.6 to 0.6 ppm) in the F_1 dimension and 5263 Hz (72 to 2 ppm) in the F_2 (carbon) dimension. Collection of 256 free induction decays (FIDs) using 2k data points in the F_2 dimension gave after zero-filling a transform size of 2k × 1k, corresponding to digital resolutions of 5.2 Hz in the F_2 dimension and 0.6 Hz in the F_1 dimension. The sine bell window was used in the F_2 dimension while exponential multiplication with 2–4 Hz line broadening was used in the F_1 dimension. In order to obtain the best proton peak shapes and chemical shifts, the matrix was transformed in the F_2 dimension, and then individual interferograms were transformed in the phase-sensitive mode with 2–4 Hz line broadening. When necessary, resolution enhancement was performed using Gaussian multiplication with a line broadening factor of −1 to −3 Hz. The cross sections corresponding to methylene carbons were analyzed as AB quartets since the decoupling does not affect geminal couplings. As previously reported (12), an extraneous peak was frequently observed in the center of the AB quartet. Occasionally, AB quartets were quite weak, and confusion of this center peak with a peak of the AB quartet could lead to an error in chemical shift. Using sample concentrations of about 0.5 M, 16–32 transients were acquired per FID, leading to a total experiment time of 2 to 3.5 hr (acquisition time 0.2 sec, recycle delay 1 sec). Proton chemical shifts determined from the HETCOR experiment are estimated to be accurate to ±0.02 ppm. ^{2}H NMR spectra were recorded at 46.1 MHz in $CHCl_3$ solution using a spectral width of 1000 Hz, an acquisition time of 0.5 sec, 1k data points (digital resolution 2 Hz), and resolution enhancement using Gaussian multiplication with a negative (−1 to −7 Hz) line broadening factor. Spectra were referenced to $CDCl_3$ at 7.24 ppm. Sample concentrations of 0.1 M produced strong peaks after a few seconds; peaks 0.2 ppm apart could be nearly baseline-resolved with resolution enhancement. ^{13}C NMR lanthanide-induced shifts (LIS) were determined by adding measured amounts of a $CDCl_3$ solution containing $Yb(fod)_3$ and recording the induced shifts from both DEPT and the normal ^{13}C NMR spectra. The LIS values, obtained by linear regression using four to seven different concentrations of $Yb(fod)_3$ (0.005 to 0.2 molar ratio), were normalized to the LIS of C-15.

Gas-liquid chromatography–mass spectrometry

Mass spectra (MS) were measured under electron impact conditions at 15, 20, and 70 eV using the following instruments: LKB-9000 (Shimadzu Corp., Kyoto), solid probe introduction; QP-1000 (Shimadzu Corp., Kyoto), solid probe and gas-liquid chromatography–MS (GLC–MS); ELQ-400 (Extrel Corp., Pittsburgh, PA), GLC-MS; Finnigan 3200 (Finnigan Corp., San Jose, CA), solid probe and GLC–MS; and JMS-HX110HF (JOEL-USA Inc., Peabody, MA), high resolution mass spectra. Capillary GLC and GLC–MS were performed using DB-1 and DB-5 fused silica columns (J & W Scientific, Inc., Rancho Cordova, CA) under the following conditions: *a*) 0.1 μm DB-5 (11 m × 0.25 mm) installed in the ELQ-400 spectrometer with temperature programming from 150°C (1 min) to 260°C at 40°C per min and with split and splitless injections in decane; *b*) 0.1 μm DB-5 (15 m and 30 m × 0.25 mm) with temperature programming from 200°C (1 min) to 280°C at 30°C per min with a glass falling needle injector (Alltech-Applied Sciences, Deerfield, IL) installed in the QP-1000 and Finnigan 3200 spectrometers; *c*) 0.1 μm DB-1 (15 m × 0.25 mm) with isothermal operation at 260°C.

The following gas chromatographs were used: Shimadzu GC9A (Shimadzu Corp., Kyoto, Japan) for both GLC and GLC–MS; Varian 3500 GC (Varian, Walnut Creek,

CA) for GLC-MS; and Sigma 2000 (Perkin Elmer, Norwalk, CT) for GLC only. In the case of the GC9A instrument, the two identical capillary columns were connected to a falling needle injector; one column was directly introduced into the EI/CI ion source via a heated interface and a second column was connected to a flame ionization detector and the additional make-up gas line. With this arrangement, parallel GLC-MS and flame ionization detection was possible.

Isotopic compositions of deuterium-labeled compounds were calculated with ~2% accuracy from spectra obtained from quadrupole mass spectrometers using intensities measured for d_0 compounds under the same conditions as a reference.

Derivatization

Acetate derivatives of the sterols were, unless stated otherwise, obtained by treatment with pyridine-acetic anhydride at room temperature for 24 hr followed by dilution with methanol and evaporation to dryness under nitrogen. Trimethylsilyl ethers (TMS) were prepared under the following conditions: *a*) *bis*-trimethylsilyl trifluoroacetamide (BSTFA)-ethyl acetate 1:1 at room temperature, 65°C or 110°C for 1 hr; *b*) BSTFA containing 1% of trimethylsilylchloride-ethyl acetate 1:1 at 65°C for 1 hr; or *c*) BSTFA-pyridine 1:1 at 65°C for 1 hr.

Derivatives of 5α-cholest-8(14)-en-3β-ol-15-one

The preparations of 5α-cholest-8(14)-en-15-one, **1** (14), 5α-cholest-8(14)-en-3β-ol-15-one (15-ketosterol), **2** (1, 15), 3β-benzoyloxy-5α-cholest-8(14)-en-15-one, **4** (15), and 5α-cholest-8(14)-en-3α-ol-15-one, **6** (16) were described previously.

3β-Acetoxy-5α-cholest-8(14)-en-15-one, 3

The 15-ketosterol **2** was acetylated as described above and recrystallized from methanol-water to give acetate **3** which melted at 135.0-135.5°C. UV, λ_{max} (hexane) 249.6 nm (ϵ = 14,840); elemental analysis, C, 78.79; H, 10.40 (calculated for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47); and ^{1}H NMR (300 MHz), 4.72 m (3α-H), 4.13 bd (7β-H), 2.36 dd (16β-H), 2.03 s (OAc), 2.0-2.15 m (16α-H and 12β-H), 1.87 m (9α-H and 2α-H), 0.99 d (J = 6.5 Hz; 21-H_3), 0.97 s (18-H_3), 0.86 d (J = 6.6 Hz; 26,27-H_6), and 0.73 s (19-H_3) (see also ^{1}H and ^{13}C NMR data in Table 4).

3α-Acetoxy-5α-cholest-8(14)-en-15-one, 7

5α-Cholest-8(14)-en-3α-ol-15-one **6** (45.2 mg) was acetylated by treatment with a mixture of acetic anhydride (1 ml) and pyridine (1 ml) for 3 hr at 45°C. The reaction mixture was diluted with water (100 ml) to give the crude acetate (48.9 mg), which was purified by preparative TLC using methylene chloride as solvent. The resulting acetate (44.4 mg) was recrystallized twice from methanol to give pure **7** (35.4 mg) which melted at 162.5-164.0°C. UV, λ_{max} (hexane) 250 nm (ϵ = 14,890); elemental analysis, C 78.61; H 10.51 (calculated for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47); high resolution MS on molecular ion, 442.3447 (calculated for $C_{29}H_{46}O_3$: 442.3447); ^{1}H NMR (300 MHz), 5.04 t (J = 2.6 Hz; 3β-H), 4.14 ddd (J = 14.2, 4.0, and 2.0 Hz; 7β-H), 2.36 dd (J = 18.6 and 7.7 Hz; 16β-H), 2.06 (OAc), 1.95-2.15 m (3 protons), 1.00 d (J = 6.4 Hz; 21-H_3), 0.97 s (18-H_3), 0.86 d (J = 6.6 Hz; 26,27-H_6), and 0.70 s (19-H_3) (see also ^{13}C NMR data in Table 4).

5α-Cholest-8(14)-ene-3,15-dione, 9

The 15-ketosterol **2** (18.50 g, 46.2 mmol) in methylene chloride (120 ml) was added to a solution of chromium trioxide (10 g) in acetic acid (300 ml) and pyridine (100 ml). The reaction was carried out under nitrogen in an ice bath. After 4 hr, the reaction mixture was warmed up to ~15°C. When none of the starting material was detected by TLC analysis (solvent system, toluene-ethyl acetate 10:1; substrate **2** R_f 0.25, product **9** R_f = 0.60), isopropanol (20 ml) and water (1000 ml) were added. The mixture was extracted with hexane (600, 125, 125 ml) and the combined extract was filtered through alumina (activity I; 3 cm × 5 cm). The product (homogeneous by TLC) was recrystallized from methanol-methylene chloride to give diketone **9** (16.08 g; 87% yield) which melted at 145-146.0°C (literature, 145.5-146.0°C (17,18)). UV, λ_{max} (hexane) 248.4 nm (ϵ = 14,470), ^{1}H NMR (300 MHz), 4.19 m (7β-H), 2.36 dd (16β-H), 2.08 dd (16α-H), 1.01 d (21-H_3), 1.01 s (18-H_3), 0.93 s (19-H_3), and 0.87 d (26,27-H_6).

[7α,9α,16,16-$^2H_4$]5α-Cholest-8(14)-en-3β-ol-15-one (2-d_4)

The 15-ketosterol **2** (4.15 g) in CH_3OD (30 ml), NaOD in D_2O (8 ml; prepared from sodium metal, 1 g, and D_2O, 15 ml) and pyridine (50 ml) were heated at 80°C overnight under an atmosphere of nitrogen. The crude product, recovered by extraction with methylene chloride, was recrystallized twice from methanol-water- hexane to give deuterated **2** (1.449 g; 35% yield) which melted at 149-151°C (reported for the unlabeled 15-ketosterol **2**, 147.5-149.0°C (1)). ^{1}H NMR (300 MHz), 4.11 bs (residual 7β-H signal), 3.64 m (3α-H), 2.10 dt (J = 12.5 and 3.5 Hz; 12β-H), 0.99 d (J = 6.5 Hz; 21-H_3), 0.97 s (18-H_3), 0.86 d (J = 6.6 Hz, 26,27-H_6), and 0.71 s (19-H_3); elemental analysis, C, 79.89; H, 10.79 (calculated for $C_{27}H_{40}D_4O_2$: C, 80.15; H, 10.96). Analysis of the isotopic composition by MS showed: d_2:4%, d_3:23%, d_4:58%, and d_5:15%.

[7α,9α,16,16-$^2H_4$]3β-Acetoxy-5α-cholest-8(14)-en-15-one (3-d_4)

[7α,9α,16,16-$^2H_4$]5α-Cholest-8(14)-en-3β-ol-15-one (**2**-d_4), from above, was treated with acetic anhydride-pyridine as described previously to give acetate **3**-d_4, which melted at

135.0–135.5°C. ^{1}H NMR (in $CDCl_3$, 300 MHz), 4.72 m (3α-H), 4.10 bs (residual 7β-H signal), 2.09 m (12β-H), 2.02 s (OAc), 1.00 d (J = 6.5 Hz; 21-H_3), 0.99 s (18-H_3), 0.86 d (J = 6.6 Hz; 26,27-H_6), and 0.73 s (19-H_3).

[7,7,9α,16,16-$^2H_5$]3β-Acetoxy-5α-cholest-8(14)-en-15-one (3-d_5)

The 15-ketosterol **2** (3.94 g) in carbon tetrachloride (25 ml) was treated with CH_3COOD (25 ml) containing 5% DCl (prepared by hydrolysis of acetic anhydride–acetyl chloride with D_2O) overnight at room temperature. The solvents were removed under reduced pressure and the same treatment was repeated once more. The resulting acetate **3** showed, by probe MS analysis, the following isotopic composition: d_4:19% and d_5:81%. This product, containing only traces of other components according to TLC analysis (solvent system, hexane–ethyl acetate 7:3; R_f of **3**, 0.82; R_f of by-products, 0.67, 0.55, 0.42, 0.22), was recrystallized twice from methanol–water to give the acetate **3** (3.80 g, 86% yield) which melted at 135.0–135.5°C. Analysis of its isotopic composition by probe MS showed d_3: <0.5%, d_4:23%, and d_5:77%. ^{1}H NMR (300 MHz), 4.73 m (3α-H), 4.12 s (7β-H, integration showed 4% of one-proton intensity), 2.11 dt (12β-H), 2.02 s (OAc), 1.86 bd (2α-H), 0.99 d (21-H_3), 0.97 s (18-H_3), 0.86 d (26,27-H_6), 0.73 s (19-H_3); ^{13}C NMR: 26 singlets and three multiplets of ^{13}C coupled with deuterium at 50.11 t (C-9), 41.91 m (C-16) and 26.95 m (C-7).

[7,7,9α,16,16-$^2H_5$]5α-Cholest-8(14)-en-3β-ol-15-one (3-d_5)

The above 15-ketosterol acetate d_5, **3**-d_5 (0.63 g) in CH_3OD (10 ml), D_2O (5 ml), and tetrahydrofuran (5 ml) was treated with potassium *tert*-butoxide (0.5 g) under nitrogen atmosphere for 3 hr at ~50°C. After removal of the solvents under reduced pressure, the residue was dissolved in ethyl ether and washed with brine. The solid residue, obtained upon evaporation of the solvent, was recrystallized twice from methanol–water to give 15-ketosterol **2**-d_5 (0.43 g, 75% yield) which melted at 147.5–148.0°C. The isotopic composition (measured by probe MS) showed d_4:20% and d_5:80%.

[2,2,3α,4,4,7,7,9α,16,16-$^2H_{10}$]5α-Cholest-8(14)-en-3β-ol-15-one-(2-d_{10})

Sample 1. The 3,15-diketone **9** (5.14 g) was treated four times with a mixture of carbon tetrachloride (25 ml) and CH_3COOD containing 5% DCl (50 ml first treatment and 25 ml for the second, third, and fourth treatments) for 12, 4, 20, and 48 hr, respectively, at room temperature. After each treatment the mixture was evaporated under reduced pressure and residual acetic acid was removed by two further evaporations with carbon tetrachloride. Analysis by TLC (solvent system, hexane–ethyl acetate 7:3) showed a mixture of products (R_f values of 0.52 (corresponding to that for **9**), 0.60, 0.70, and 0.78). The crude product was dissolved in tetrahydrofuran (100 ml) and treated with a solution of $NaBD_4$ in D_2O at room temperature until all of the starting material **9** was consumed (other by-products remained unchanged). Two major new products (**2** and **6**, R_f 0.16 and 0.20, respectively) were separated from less polar components by alumina (activity III) column chromatography using elution with methylene chloride–hexane 3:7, methylene chloride, and methylene chloride–ethyl ether 7:3. Polar fractions, after two recrystallizations from methanol–water yielded the homogenous 15-ketosterol **2**-d_{10} (2.14 g, 40% yield) which melted at 145.0–146.0°C. Analysis of its isotopic composition by probe MS showed d_8:11%, d_9:35%, and d_{10}:54%. [2]

Sample 2. 3,15-Diketone **9** (4.01 g) in carbon tetrachloride (50 ml) was treated twice with CH_3COOD containing 2.5% DCl for 20 hr at room temperature. The intermediate diketone **9** showed the following isotopic composition when analyzed by probe MS: first treatment, d_4:1%, d_5:3%, d_6:10%, d_7:25%, d_8:33%, and d_9:22%; second treatment, d_6:2%, d_7:11%, d_8:36%, and d_9:51%. Diketone **9**-d_9 was separated from minor, less polar by-products by two recrystallizations from methanol–water (2.59 g, 64% yield). This sample of diketone **9**-d_9 was dissolved in tetrahydrofuran (100 ml) and reduced with $NaBD_4$ in D_2O as described above. Products were separated on alumina using the same elution sequence. Recrystallization from methanol–water gave homogeneous 15-ketosterol **2**-d_{10} (2.03 g, 79% yield) which melted at 146.5–147.0°C. Analysis of isotopic composition by probe MS showed d_7:2%, d_8:11%, d_9:35%, and d_{10}:52%. An additional amount (0.92 g) of 15-ketosterol **2**-d_{10} was obtained from the combined mother liquor of the diketone **9**-d_9 by reduction with $NaBD_4$ followed by chromatography and crystallization. The total yield of **2**-d_{10} calculated from the unlabeled 3,15-diketone **9** was 72%.

[2,2,3α,4,4,-$^2H_5$]3β-Acetoxy-5α-cholest-8(14)-en-15-one, 3-d_5

The 15-ketosterol **2**-d_9 (3.03 g) in carbon tetrachloride (50 ml for the first treatment and 25 ml for the second and third treatments) was treated twice with 3% HCl in acetic acid (50 ml, prepared by the hydrolysis of acetyl chloride

[2]It should be noted that complete deuteration (i.e., 100% d_{10} species) should not be anticipated under the conditions employed. In the case of the deuteration of compound **9** (with 9 hydrogens available for exchange and with the subsequent introduction of an additional atom of deuterium in the borodeuteride reduction of the 3-ketone function), deuteration to the extent of 95% at each of the 10 sites would give only 60% of a d_{10} species. Thus, the observed isotopic composition of 54% d_{10} species is compatible with very high levels of deuteration (i.e., 90% that expected for 95% deuteration at each of the 10 sites).

TABLE 1. Electron impact mass spectra of derivatives of 5α-cholest-8(14)-en-15-one (*1*)

Fragment Ion, *m/z* (% rel. int.)	1[a]	2[a]	2-d_5[b,c]	2-d_5[b,d]	3[b]	3-d_5[b,d]
$M^{+\cdot}$	384 (71)[e]	400 (87)[e]	405 (100)	405 (100)	442 (100)[e]	447 (100)
M-CH_3	369 (61)[e]	385 (17)[e]	390 (25)	390 (26)	427 (19)	432 (19)
M-H_2O	366 (7)	382 (15)[e]	387 (18)	385,387 (11,11)	424 (9)	427 (10)
M-CH_3-H_2O	351 (5)	367 (26)[e]	372,371 (43,17)	372 (45)	409 (4)	412 (2)
M-ROH					382 (23)	387 (5)
M-ROH-CH_3					367 (77)	372 (54)
M-H_2O-H_2O		364 (1)				
M-H_2O-ROH					364 (4)	367 (1)
M-H_2O-ROH-CH_3		349 (1)	354 (2)		349 (5)	
M-85	299 (2)	315 (1)				
M-85-H_2O	281 (3)	297 (3)	302 (5)	301 (3)	339 (4)	342,343 (2,2)
M-SC	271 (34)	287 (22)[e]	292 (23)	292 (33)	329 (15)[e]	334 (23)
M-SC-H_2O	253 (100)[e]	269 (100)[e]	274 (97)	272,273 (45,45)	311 (38)[e]	314 (29)
M-SC-ROH					269 (42)[e]	274,273 (33,16)
M-SC-H_2O-H_2O		251 (28)	256,255 (19,10)	254,255 (10,18)		
M-SC-H_2O-ROH					251 (81)[e]	255 (27)
	276 (26)[e]	276 (7)[e]		280,281 (6,9)	276 (7)	281,280 (3,3)
	261 (79)[e]	261 (18)[e]	261,262 (12,9)	266 (15)	261 (25)[e]	266 (8)
M-SC-28	243 (12)	259 (6)	264 (9)	264 (12)	301 (18)[e]	306,305 (5,4)
M-SC-28-H_2O		241 (5)	245,246 (3,6)	244,245 (3,4)		
M-SC-28-ROH					241 (19)	245,246 (4)

	3-d_5[b,c]	4[f]	5[a]	5-d_5[a,c]	5-d_5[a,d]
$M^{+\cdot}$	447 (100)	504 (100)	472 (80)	477 (100)	477 (100)
M-CH_3	432 (25)	489 (6)	457 (17)	462 (22)	462 (15)
M-H_2O	429 (14)	486 (5)	454 (7)	459 (9)	457 (6)
M-CH_3-H_2O	414 (1)		439 (2)	444 (1)	
M-ROH	386 (20)	382 (5)	382 (20)	387 (22)	387 (17)
M-ROH-CH_3	372,371 (64,59)	367 (16)	367 (78)	372 (95)	372 (70)
M-H_2O-H_2O					
M-H_2O-ROH	369 (7)		364 (5)	369 (8)	
M-H_2O-ROH-CH_3	354 (5)		349 (7)	354 (7)	353,354 (3)
M-85					
M-85-H_2O	344 (6)				
M-SC	334 (18)	391 (6)	359 (7)	364 (9)	364 (9)
M-SC-H_2O	316 (46)	373 (7)	341 (24)	346 (34)	344,345 (20,20)
M-SC-ROH	274,273 (35,43)	269 (12)	269 (36)	274 (45)	274 (28)
M-SC-H_2O-H_2O					
M-SC-H_2O-ROH	256,255 (50,68)	251 (12)	251 (100)	256 (87)	254,255,256 (27,28,20)
		276 (3)	276 (16)	276,277 (10,10)	280,281 (8,13)
	261,262 (24,19)	261 (3)	261 (36)	261,262 (18,22)	266 (17)
M-SC-28	306 (18)		331 (5)	336 (6)	333 (3)
M-SC-28-H_2O					
M-SC-28-ROH	245,246 (19)	241 (1)	241 (15)	246 (18)	243-246 (3-5)

in acetic acid) for 24, 24, and 48 hr. Analysis of its isotopic composition by probe MS showed: first exchange, d_4:7%, d_5:33%, d_6:49%, and d_7:11%; second exchange, d_3: ~1%, d_4:17%, d_5:53%, d_6:27%, and d_7: ~1%; and, after the third exchange, d_3:4%, d_4:28%, d_5:64%, and d_6:4%. This acetate was purified on alumina in hexane–methylene chloride stepwise gradient elution. After recrystallization from methanol–water it was obtained in 46% yield and melted at 132.5–133.5°C.

[2,2,3α,4,4,-$^2H_5$]5α-Cholest-8(14)-en-3β-ol-15-one (2-d_5)

Sample 1. The crude product of the deuteration of diketone **9** (obtained as described for sample 2 of **9**-d_9 but without further purification by recrystallization) was reduced with $NaBD_4$ as described above. The solid residue, obtained after removal of solvents at reduced pressure, was refluxed for 5 days under nitrogen atmosphere with 2% KOH in ethanol. The products were separated by column chromatography on silica gel, and the 15-ketosterol **2**-d_5 obtained therefrom was additionally purified by preparative TLC (solvent system, toluene–ethyl acetate 13:2, three developments) and recrystallization from methanol–water to give **2**-d_5 which melted at 149–150°C. Treatment with acetic anhydride-pyridine gave the corresponding acetate. Analysis of its isotopic composition by GLC–MS showed d_3:3%, d_4:28%, and d_5:72%. ^1H NMR (300 MHz), 4.71 s (residual 3α-H sig-

TABLE 1. *Continued*

	6[a]	7[a]	8[a]	8-d_5[a,c]	9[b]	9[f]
M+	400 (26)[e]	442 (41)	472 (65)	477 (87)	398 (100)[e]	398 (100)
M-CH_3	385 (7)	427 (6)	457 (5)	462 (8)	383 (79)	383 (47)
M-H_2O	382 (92)	424 (4)	454 (5)	459 (7)	380 (10)	380 (9)
M-CH_3-H_2O	367 (90)	409 (1)	439 (1)	444 (1)	365 (9)	365 (4)
M-ROH		382 (79)	382 (32)	387 (42)		
M-ROH-CH_3		367 (95)	367 (80)	372 (100)		
M-H_2O-H_2O	364 (11)					
M-H_2O-ROH		364 (9)	364 (6)	369 (9)		
M-H_2O-ROH-CH_3	349 (9)	349 (11)	349 (7)	354 (7)		
M-85					313 (2)	
M-85-H_2O	297 (6)	339 (4)			295 (4)	
M-SC	287 (9)[e]	329 (2)	359 (3)	364 (4)	285 (77)[e]	285 (55)
M-SC-H_2O	269 (68)[e]	311 (12)	341 (15)	346 (23)	267 (74)[e]	267 (32)
M-SC-ROH		269 (74)	269 (40)	274 (60)		
M-SC-H_2O-H_2O	251 (100)					
M-SC-H_2O-ROH		251 (100)	251 (100)	256 (88)		
	276 (10)	276 (7)	276 (28)	276,277 (23,23)		
	261 (31)	261 (24)	261 (47)	261,262 (24,32)	261 (9)[e]	261 (3)
M-SC-28	259 (8)	301 (5)	331 (3)	336 (5)	257 (25)	257 (6)
M-SC-28-H_2O	241 (22)					
M-SC-28-ROH		241 (29)	241 (18)	246 (22)		

[a]Quadrupole instrument, 20 eV.
[b]Quadrupole instrument, 70 eV.
[c]Labeled at C-2,2,3,4,4.
[d]Labeled at C-7,7,9,16,16.
[e]Confirmed by HRMS, see Table 2.
[f]Magnetic sector instrument, 15 eV.
SC, the alkyl side chain (C_8H_{17}) of the sterol; RO, the substituent at C-3.

nal), 4.12 m (7β-H), 2.35 dd (16β-H), 2.0–2.15 m (16α-H and 12β-H), 2.03 s (OAc), 1.87 dd (9α-H), 1.72 and 1.21 (ABq of 1-H_2), 1.00 d (J = 6.4 Hz, 21-H_3), 0.97 s (18-H_3), 0.86 d (J = 6.6 Hz, 26,27-H_6), 0.73 s (19-H_3).

Sample 2.[3] 5α-cholest-8(14)-ene-7α,15α-diol-3-one was deuterated at C-2 and C-4 under alkaline conditions. The crude product was reduced with $NaBD_4$ in isopropyl alcohol-D_2O to give [2,2,3α,4,4-$^2H_5$]5α-cholest-8(14)-ene-3β,7α,15α-triol and its 3α-epimer. This mixture, without separation, was treated with HCl–ethanol as described before (8,14), to give two 3-epimeric 15-ketosterols **2**-d_5 and **6**-d_5. The principal product, [2,2,3α,4,4-$^2H_5$]5α-cholest-8(14)-en-3β-ol-15-one, was isolated by preparative TLC (solvent system, hexane–ethyl acetate 7:3; developed three times at 0°C. It was homogeneous by GLC and TLC and melted at 145.5–146.5°C after recrystallization from methanol–water. GLC–MS of the corresponding TMS ether showed the following isotopic composition: $d_3 < 1\%$, d_4:12%, and d_5:87%.

RESULTS AND DISCUSSION

Introduction of deuterium

The 15-ketosterol **2** labeled with deuterium in ring A at positions 2,2,3,4,4 was prepared by two different methods. The first was based on the exhaustive deuterium exchange of diketone **9** under acidic conditions, followed by reduction with sodium borodeuteride, and then by removal of deuterium label from positions 7, 9, and 16 under either alkaline or acidic conditions. The second method was analogous to that previously employed for the preparation of tritiated 5α-cholest-8(14)-en-3β-ol-15-one **2** (19) and involved the exchange of positions 2 and 4 under basic conditions in 5α-cholest-8(14)-ene-7α,15α-diol-3-one, followed by its sodium borohydride reduction and acid-induced rearrangement to give the 8(14)-en-15-one system. Three other deuterated species of 15-ketosterol **2** were prepared as follows: *a*) by the direct exchange of the parent compound in the alkaline medium to give [7α,9α,16,16-d_4]-**2**; *b*) by the direct exchange in the acidic medium to give (7,7,9α,16,16-d_5]-**2**; and *c*) by the exchange of the 3,15-diketone in the acidic medium followed by the sodium borodeuteride reduction to give [2,2,3α,4,4,7,7,9α,16,16-d_{10}]-**2**.

Deuterium exchange, both in basic solutions (pyridine, CH_3OD-NaOD) as well as in acidic medium (CH_3 COOD–DCl) indicated that the complete deuteration of all five enolizable positions in the α,β-unsaturated ketones

[3]This reaction was performed by Dr. Gary T. Emmons.

TABLE 2. High resolution mass spectral data of 5α-cholest-8(14)-en-3β-ol-15-one and its major derivatives

Compound	m/z (% Intensity) Measured		Calculated	Composition	
1	386.3411	(3)	386.3459	$C_{25}{}^{13}C_2H_{44}O$	
	385.3414	(31)	385.3425	$C_{26}{}^{13}CH_{44}O$	
	384.3431	(100)	384.3392	$C_{27}H_{44}O$	(M^+)
	370.3210	(5)	370.3191	$C_{25}{}^{13}CH_{41}O$	
	369.3203	(19)	369.3157	$C_{26}H_{41}O$	($M-CH_3$)
	276.2481	(6)	276.2453	$C_{19}H_{32}O$	
	262.2217	(1)	262.2252	$C_{17}{}^{13}CH_{29}O$	
	261.2217	(8)	261.2218	$C_{18}H_{29}O$	
	253.1942	(9)	253.1956	$C_{19}H_{25}$	($M-SC-H_2O$)[a]
	175.0970	(1)	175.1123	$C_{12}H_{15}O$	
	161.0969	(1)	161.0966	$C_{11}H_{13}O$	
	136.0860	(1)	136.0888	$C_9H_{12}O$	
2	402.3418	(5)	402.3408	$C_{25}{}^{13}C_2H_{44}O_2$	
	401.3360	(29)	401.3375	$C_{26}{}^{13}CH_{44}O_2$	
	400.3315	(100)	400.3341	$C_{27}H_{44}O_2$	(M^+)
	399.3243	(1)	399.3263	$C_{27}H_{43}O_2$	
	386.3174	(1)	386.3140	$C_{25}{}^{13}CH_{41}O_2$	
	385.3115	(6)	385.3107	$C_{26}H_{41}O_2$	($M-CH_3$)
	383.3311	(1.5)	383.3269	$C_{26}{}^{13}CH_{42}O$	
	382.3272	(4)	382.3236	$C_{27}H_{42}O$	($M-H_2O$)
	368.3088	(1)	368.3079	$C_{26}H_{40}O$	
	368.3022	(2)	368.3035	$C_{25}{}^{13}CH_{39}O$	
	367.2984	(9)	367.3001	$C_{26}H_{39}O$	($M-CH_3-H_2O$)
	288.2015	(1)	288.2045	$C_{18}{}^{13}CH_{27}O_2$	
	287.2036	(5)	287.2001	$C_{19}H_{27}O_2$	($M-SC$)
	276.2453	(1)	276.2453	$C_{19}H_{32}O$	
	270.1942	(3)	270.1939	$C_{18}{}^{13}CH_{25}O$	
	269.1909	(12)	269.1905	$C_{19}H_{25}O$	($M-SC-H_2O$)
	261.2218	(2)	261.2218	$C_{18}H_{29}O$	
	259.1696	(1)	259.1698	$C_{17}H_{23}O_2$	
	175.1162	(0.4)	175.1123	$C_{12}H_{15}O$	
	136.0907	(0.2)	136.0966	$C_9H_{12}O$	
3	443.3490	(27)	443.3481	$C_{28}{}^{13}CH_{46}O_3$	
	442.3405	(100)	442.3447	$C_{29}H_{46}O_3$	(M^+)
	427.3316	(5)	427.3212	$C_{28}H_{43}O_3$	($M-CH_3$)
	367.2959	(12)	367.3001	$C_{26}H_{39}O$	($M-AcOH-CH_3$)
	329.2220	(3)	329.2117	$C_{21}H_{29}O_3$	($M-SC$)
	312.2096	(2)	312.2045	$C_{20}{}^{13}CH_{27}O_2$	
	311.2014	(6)	311.2011	$C_{21}H_{27}O_2$	($M-SC-H_2O$)
	301.1830	(1)	301.1804	$C_{19}H_{25}O_3$	($M-SC-28$)
	269.1909	(5)	269.1905	$C_{19}H_{25}O$	($M-SC-AcOH$)
	261.2299	(1)	261.2218	$C_{18}H_{29}O$	
	251.1813	(5)	251.1800	$C_{19}H_{23}$	($M-SC-H_2O-AcOH$)
	175.1155	(1)	175.1123	$C_{12}H_{15}O$	
	161.0978	(1)	161.0966	$C_{11}H_{13}O$	
	159.1197	(1)	159.1174	$C_{12}H_{15}$	

2 and **9** was relatively difficult. Thus, despite several exchanges with fresh portions of deuterated medium, it was impossible to completely remove the d_4 species when **2** was reacted. Similarly, in the deuteration of diketone **9**, substantial amounts of the d_8 species remained. ^1H, ^2H, and ^{13}C NMR (see discussion below) revealed that positions 9α, 16α, 16β, and 7α were efficiently deuterated, whereas the deuterium incorporation at 7β was only partial. This difference can be ascribed to the protonation-deprotonation of the intermediate enolate being favored from the α-face, thus lowering the extent of deuteration of the 7β-proton.[4]

The complete removal of deuterium from positions 7, 9 and 16, required in the first synthetic route to ring A labeled **2** from d_{10}-**2**, was slow and required boiling for 5 days in 2% potassium hydroxide in ethanol. Alternatively, repeated exchanges with HCl–acetic acid at room temperature, resulting in the acetate **3**-d_5, were employed. Once

[4]The difficulties in the full deuteration at C-7 are also consistent with our observation that 5α-cholest-8-en-3β-ol-15-one is the major deconjugation product formed from 5α-cholest-8(14)-en-3β-ol-15-one **2** under acidic conditions. The Δ^7 isomer could be detected only as a trace component of the reaction mixture (unpublished data).

TABLE 2. *Continued*

Compound	*m/z* (% Intensity) Measured		Calculated	Composition	
6	401.3322	(38)	401.3375	$C_{26}{}^{13}CH_{44}O_2$	
	400.3326	(100)	400.3341	$C_{27}H_{44}O_2$	(M^+)
	382.3326	(6)	382.3236	$C_{27}H_{42}O_2$	($M-H_2O$)
	367.3022	(11)	367.3001	$C_{26}H_{39}O$	($M-H_2O-CH_3$)
	287.1974	(3)	287.2011	$C_{19}H_{27}O_2$	(M-SC)
	269.1900	(27)	269.1905	$C_{19}H_{25}O_2$	($M-SC-H_2O$)
9	400.3249	(3)	400.3252	$C_{25}{}^{13}C_2H_{42}O_2$	
	399.3226	(36)	399.3218	$C_{26}{}^{13}CH_{42}O_2$	
	398.3181	(100)	398.3185	$C_{27}H_{42}O_2$	(M^+)
	397.3181	(1)	397.3107	$C_{27}H_{41}O_2$	
	384.3017	(12)	384.2984	$C_{25}{}^{13}CH_{39}O_2$	
	383.2978	(52)	383.2950	$C_{26}H_{39}O_2$	($M-CH_3$)
	381.3104	(3)	381.3113	$C_{26}{}^{13}CH_{40}O$	
	380.3083	(6)	380.3079	$C_{27}H_{40}O$	($M-H_2O$)
	295.2103	(1)	295.2062	$C_{21}H_{27}O$	($M-85-H_2O$)
	286.1904	(8)	286.1888	$C_{18}{}^{13}CH_{25}O_2$	
	285.1853	(48)	285.1855	$C_{19}H_{25}O_2$	(M-SC)
	273.2226	(2)	273.2218	$C_{19}H_{29}O$	
	268.1801	(7)	268.1782	$C_{18}{}^{13}CH_{23}O$	
	267.1761	(24)	267.1749	$C_{19}H_{23}O$	($M-SC-H_2O$)
	261.2214	(2)	261.2218	$C_{18}H_{29}O$	
	259.1664	(2)	259.1698	$C_{17}H_{23}O_2$	(M-SC-26)
	258.1635	(4)	258.1620	$C_{17}H_{22}O_2$	(M-SC-27)
	257.1564	(5)	257.1542	$C_{17}H_{21}O_2$	(M-SC-28)
	253.1564	(1)	253.1592	$C_{18}H_{21}O$	
	249.1621	(1)	249.1643	$C_{19}H_{21}$	
	230.1652	(2)	230.1671	$C_{16}H_{22}O$	
	215.1468	(2)	215.1436	$C_{15}H_{19}O$	
	175.1112	(5)	175.1123	$C_{12}H_{15}O$	
	161.0985	(2)	161.0966	$C_{11}H_{13}O$	

[a]SC, the alkyl side chain (C_8H_{17}) of the sterol.

more, elimination of the 7β-deuterium was responsible for the low efficacy of this back-exchange. Under neutral conditions, including chromatography on alumina, the deuterium label at positions 7, 9, and 16 was stable. A small (~4–5%) loss of deuterium occurred when compounds labeled at these positions were recrystallized from boiling methanol.

The instability of **2** and **9** (especially under basic conditions in the presence of air) rendered the forcing conditions for the deuterium exchange relatively inconvenient. Thus, in the presence of air, slow oxidation to several polar products containing an additional oxygen atom was observed. In addition, in the course of the deuteration of diketone **9** with CH_3COOD–DCl at room temperature, a dimeric product was formed. This product (or products) slowly accumulated in the multiply exchanged mixture. Its mass spectrum (solid probe inlet system) showed prominent ions at *m/z* 794, 795, and 796, ions which correspond to those expected as a result of the aldol condensation of two molecules of the d_9-3,15-diketone with the loss of a molecule of D_2O. In order to lower the extent of dimerization, the deuteration of diketone **9** was carried out using an approximately 20-fold excess of deuterated acetic acid containing 2.5% of DCl. Two consecutive exchanges at room temperature were sufficient for extensive exchange.

Mass spectral fragmentation of 5α-cholest-8(14)-en-15-one and its derivatives

Detailed analysis of the mass spectrum of the 15-ketosterol **2** was required for the interpretation of the mass spectra of a number of derivatives of **2**. In addition, information regarding the origin of a number of fragment ions in the mass spectra of **2** and of its derivatives was required for several metabolic studies in progress in our laboratory. Accordingly, a series of deuterated species of the 15-ketosterol **2** and of a number of its derivatives (with varying substitution at C-3) were prepared. The low resolution electron impact mass spectra of these compounds were studied **(Table 1)**. In addition, the high resolution mass spectra (HRMS) of compounds **1**, **2**, **3**, **6**, and **9** were obtained **(Table 2)** to provide critical information in support of ion assignments based upon analyses of the low resolution spectra of the d_0-compounds and

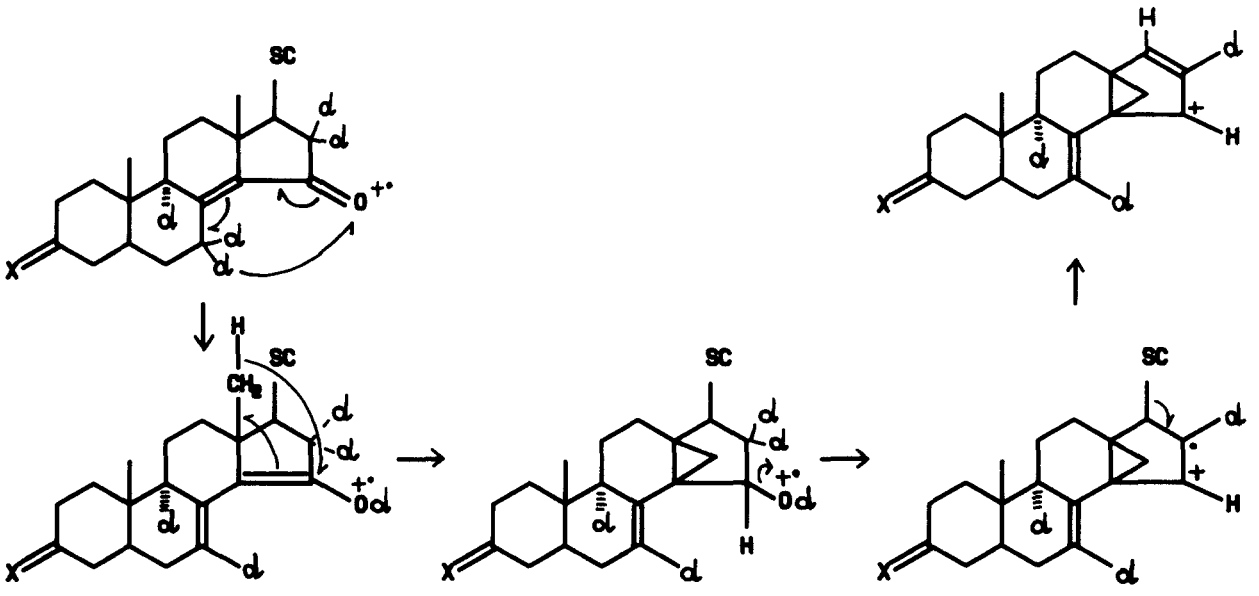

Fig. 2. Proposed mode of origin of fragment ions corresponding to M-H_2O and M-H_2O-alkyl side chain (C_8H_{17}) in the mass spectra of derivatives of 5α-cholest-8(14)-en-15-one (**1**) and its derivatives.

their deuterated analogs. The low resolution mass spectrum of **1**, prepared by an independent method, has been presented previously by Midgley and Djerassi (20).

Ions in the molecular ion region of **2** and its derivatives were, for the most part, relatively easy to assign based upon analyses of the low resolution spectra of the d_0 compounds and their deuterium-labeled analogs coupled with the results of HRMS analyses. All compounds studied showed molecular ions in high abundance. In many cases, the molecular ions represented the base peaks in the spectra. Other ions corresponded to the elimination of a methyl radical and a molecule of water, both processes giving rise to strong metastable ions. Ions due to the elimination of a methyl radical and the C-3 substituent were very prominent in the spectra of the acetate esters and trimethylsilyl ethers of **2** and **6**.

The loss of water from **1**, the 3-deoxy analog of **2**, necessarily involves the loss of the 15-oxygen atom. Analyses of the spectra of the acetate esters of the 3β-hydroxy-15-ketosterol, i.e., **3**, and of its deuterated analogs **3**-d_5 (deuterium at 7,7,9,16,16) and **3**-d_5 (deuterium at 2,2,3,4,4) and of the trimethylsilyl ethers of the 3β-hydroxy-15-ketosterol, i.e., **5**, and of its deuterated analogs **5**-d_5 (deuterium at 7,7,9,16,16) and **5**-d_5 (deuterium at 2,2,3,4,4) suggest that the loss of water from **3** and **5** also involves the 15-oxygen atom since loss of D_2O was observed for molecules labeled with deuterium in rings B and D. One possible mechanism for this loss is presented in **Fig. 2**. No elimination of deuterium was detected in the loss of water from the molecules labeled with deuterium only in ring A.

Ions of low abundance (1–2%) were observed at M-85 in the spectra of **1**, **2**, and **9**. Ions of low abundance (3–6%) were also observed at M-85-18 in the spectra of **1**, **2**, **3**, **6**, **7**, and **9**. High retention of deuterium in the ion corresponding to M-85-18 was observed in the spectra of [2,2,3α,4,4-$^2H_5$]-**2**, [2,2,3α,4,4-$^2H_5$]-**3**, [7,7,9α,16,16-$^2H_5$]-**2**, and [7,7,9α,16,16-$^2H_5$]-**3** (Table 1). Moreover, HRMS data (Table 2) on the ion at *m/z* 295 (corresponding to M-85-18) in the spectrum of the diketone **9** was compatible with the elemental composition $C_{21}H_{27}O$, corresponding to M-C_6H_3-H_2O. The combined findings are compatible with the formation of the ion at M-85-18 by processes involving loss of a portion (C-22 through C-27) of the hydrogen-rich alkyl side chain and the elements of water. Definitive confirmation of this suggestion will require ad-

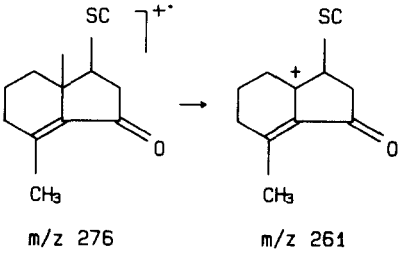

Fig. 3. Fragment ions at *m/z* 261 and 276 in the mass spectra of derivatives of 5α-cholest-8(14)-en-15-one (**1**); SC, the alkyl side chain (C_8H_{17}) of the sterol.

ditional experimentation involving derivatives of **2** at various positions in the side chain.

All of the compounds studied showed ions corresponding to M–C_8H_{17} (the alkyl side chain, SC). All of the compounds showed prominent ions corresponding to M–SC H_2O (or D_2O in the cases of **2**-d_5 (deuterium at 7,7,9, 16,16), **3**-d_5 (deuterium at 7,7,9,16,16), **5**-d_5 (deuterium at 7,7,9,16,16)). One possible mechanism for the formation of this ion is shown in Fig. 2.

Major ions in the spectra of the 3-hydroxysterols **2** and **6** were those corresponding to M–SC–H_2O–H_2O. In the spectra of the acetate esters of **2** and **6**, the benzoate ester of **2**, and the trimethylsilyl ether derivatives of **2** and **6**, major ions were those corresponding to M–SC–H_2O–ROH. Ions of lower abundance which corresponded to M–SC–28 were observed in the spectra of all compounds except **4**. The results of HRMS studies of **2**, **3**, and **9** indicated that this ion corresponds to M–SC–C_2H_4. The partial loss of deuterium in the formation of this ion in the molecules labeled with deuterium in rings B and D suggests that the C_2H_4 unit may be derived from C-16 and C-17. Ions corresponding to M–SC–28–H_2O (in the cases of the 3-hydroxysterols) and to M–SC–28–ROH (in the cases of the esters and trimethylsilyl ether derivatives of **2** and **6**) were also present in each of the compounds.

Ions at *m/z* 276 and 261 (with the exception of the absence of the ion at *m/z* 276 in the case of **9**) were significant ions in the spectra of all of the $\Delta^{8[14]}$-15-ketones (**1**–**9**). The ion at *m/z* 261 was of very high abundance (79%) in the spectrum of **1**. Determinations of the exact mass of the ion at *m/z* 276 in **1** and **2** indicated an elemental composition of $C_{19}H_{32}O$. HRMS studies of the ion at *m/z* 261 in **1**, **2**, **3**, and **9** indicated an elemental composition of $C_{18}H_{29}O$. These elemental compositions, coupled with the clear retention of the oxygen from C-15 in these ions in the spectrum of **1** and the retention of deuterium in these ions in the spectra of compounds **2** and **3** labeled with deuterium at 7,7,9,16,16 indicated that these ions were derived from rings C and D and the alkyl side chain **(Fig. 3)**. Similar conclusions, based upon low resolution MS analyses of **1** and its 7,7,9,16,16-d_5 derivative were presented previously by Midgley and Djerassi (20). One possible scheme to account for the formation of these ions was also presented (20). In the present study, partial incorporation of one deuterium atom into these ions was observed in the cases of spectra of **2**, **3**, **5**, and **8** labeled with deuterium in ring A.

The low mass fragment ions at *m/z* 136, 161, and 175, all containing one oxygen atom, were observed with variable intensities in the spectra of **1**–**9**. These ions are evidently due to a C–D fragment containing the C-15 oxygen. No attempt was made to elucidate the mode of formation of these ions.

Nuclear magnetic resonance studies of the 15-ketosterol and its derivatives

The results of previous studies of ^{13}C NMR spectra of different derivatives of 15-ketosterol **2** (21, 22) were used to corroborate the site and extent of deuteration. Additional, very illustrative data were obtained when ^{13}C NMR spectra of differently deuterated compounds were measured under a pulse sequence used to detect quaternary carbon atom signals. Thus, the spectrum of **2**-d_4, deuterated at 7α, 9α, 16α, and 16β, showed a triplet for C-9, $J_{^{13}C^{2}H}$ = 18 Hz at δ 50.23, a quartet for C-16, $J_{^{13}C^{2}H}$ = 18 Hz at δ 41.81, and a triplet (not a quartet) for C-7, $J_{^{13}C^{2}H}$ = 19 Hz at δ 27.91. **Fig. 4** presents a ^{2}H NMR spectrum of the same, partially deuterated ketosterol **2**-d_4. Comparisons of the ^{2}H NMR chemical shifts with the ^{1}H NMR shifts in **Table 3** provided additional confirmation of the deuteration sites.

Table 3 lists ^{1}H and ^{13}C NMR chemical shifts for the 15-ketosterols **1**–**3**. Also presented in Table 3 are ^{13}C NMR chemical shift data for the 3α-hydroxy-$\Delta^{8[14]}$-en-15-ketosterol **6** and its acetate derivative **7**. The ^{13}C NMR assignments were made by determining the carbon multiplicities from a series of DEPT spectra (10) and matching the observed values with reported assignments for close analogs. These assignments were confirmed by measuring lanthanide-induced shifts (LIS) using Yb(fod)$_3$ and by determining the chemical shifts of the attached protons from a two-dimensional NMR ^{1}H-^{13}C hetero-

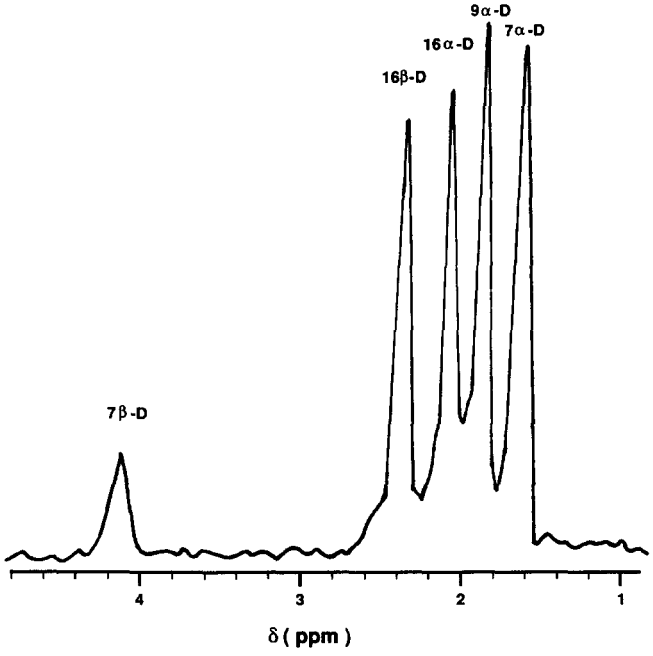

Fig. 4. Computer-enhanced deuterium NMR spectrum of [7α,9α, 16,16-$^{2}H_5$]5α-cholest-8(14)-en-3β-ol-15-one (**2**-d_4).

TABLE 3. NMR data of 5α-cholest-8(14)-en-15-one and its derivatives[a]

Carbon Atom	1		2			3		6	7
	^{13}C	LIS[b]	^{1}H[c]	^{13}C	LIS[b]	^{1}H[c]	^{13}C	^{13}C[d]	^{13}C[d]
1	38.4	0.07	1.72, 1.20	36.5	0.22	1.74, 1.25	36.2	31.7	32.3*
2	21.9	0.05	1.85, 1.38	31.0	0.46	1.86, 1.40	27.1	28.7	25.8
3	26.4	0.04	3.64	70.8	0.96	4.73	73.1	66.1	69.6
4	28.6	0.05	1.68, 1.28	37.7	0.47	1.71, 1.35	33.5	35.4	32.4*
5	46.2	0.09	1.41	44.1	0.24	1.47	43.8	38.5	39.4
6	29.7	0.14	1.48, 1.34	29.1	0.18	1.48, 1.40	28.9	29.2	28.9
7	27.8	0.33	4.13, 1.58	27.5	0.27	4.14, 1.57	27.4	27.7	27.6
8	151.7	0.42		150.7	0.35		150.1	151.1	150.7
9	51.3	0.19	1.86	50.8	0.19	1.88	50.7	50.9*	50.69**
10	39.7	0.11		38.7	0.22		38.6	39.38	39.0
11	19.3	0.14	1.65, 1.53	19.5	0.14	1.65, 1.53	19.4	19.18	19.18
12	37.1	0.16	2.11, 1.25	36.9	0.13	2.10, 1.25	36.8	37.0	36.9
13	42.6	0.26		42.5	0.22		42.4	42.6	42.52
14	139.9	0.49		140.2	0.40		140.3	140.1	140.2
15	208.2	1.00		208.2	1.00		208.0	208.2	208.1
16	42.5	0.56	2.36, 2.06	42.4	0.43	2.35, 2.05	42.3	42.5	42.47
17	50.8	0.24	1.46	50.7	0.20	1.46	50.6	50.8*	50.70**
18	18.8	0.13	0.972 (s)	18.7	0.15	0.970 (s)	18.7	18.8	18.8
19	12.8	0.11	0.715 (s)	12.9	0.19	0.731 (s)	12.7	11.8	11.9
20	34.5	0.11	1.57	34.4	0.09	1.58	34.4	34.5	34.5
21	19.2	0.13	0.998 (d, 6.4)	19.2	0.06	0.998 (d, 6.3)	19.1	19.22	19.20
22	35.8	0.05	1.33, 1.08	35.8	0.04	1.33, 1.08	35.7	35.8	35.8
23	23.5	0.03	1.33, 1.18	23.4	0.02	1.32, 1.19	23.4	23.5	23.5
24	39.4	0.00	1.15, 1.11	39.3	0.00	1.14, 1.11	39.3	39.36	39.3
25	28.0	0.00	1.51	27.9	−0.01	1.51	27.9	28.0	27.9
26	22.5	0.00	0.861 (d, 6.6)	22.5	−0.01	0.860 (d, 6.6)	22.5	22.5	22.5
27	22.7	0.00	0.863 (d, 6.6)	22.7	−0.01	0.862 (d, 6.6)	22.7	22.7	22.7

[a] NMR data measured using an IBM AF300 NMR Spectrometer at 300.1 MHz (^{1}H) and 75.5 MHz (^{13}C) in $CDCl_3$ solution (approximately 0.1 M).
[b] Lanthanide-induced shifts (Yb(fod)$_3$) relative to an assigned value of unity for C-15.
[c] Values (± 0.02 ppm) for methylene and methine protons extracted from heteronuclear ^{1}H-^{13}C shift correlation data. Multiplicities and coupling constants (in Hz) of methyl protons are given in parentheses. Spectra were measured using 128 or 256 increments and a digital resolution of 0.002 or 0.004 ppm in the F_1 (proton) dimension.
[d] Values marked with asterisks may be interchanged.

nuclear correlation experiment (23, 24). The ketone **1** could be assigned almost entirely by matching chemical shifts with those reported (25) for carbons in ring A of 5α-cholestan-15-one and for the remaining carbons in **2** (21, 22). Acetate **3** was assigned by matching observed peaks with those reported for **2**. Apart from the expected 3 ppm downfield shift for C-3 and the 4 ppm upfield shifts of C-2 and C-4 in **3**, the spectra of **2** and **3** were virtually identical.

The HETCOR experiment, which correlates each carbon atom with its attached proton(s), was carried out with proton decoupling so that vicinal ^{1}H–^{1}H couplings were nearly eliminated. Because geminal couplings were not eliminated, the exact chemical shifts of the two methylene protons were calculated from AB quartets. Proton peaks in cross sections of the HETCOR spectrum corresponding to methylene carbons were much weaker and broader than in cross sections corresponding to methyl and methine carbons. Consequently, methyl and methine chemical shifts were considerably more accurate.

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REFERENCES

1. Schroepfer, G. J., Jr., E. J. Parish, H. W. Chen, and A. A. Kandutsch. 1977. Inhibition of sterol biosynthesis in L cells and mouse liver cells by 15-oxygenated sterols. *J. Biol. Chem.* **252:** 8975–8980.

2. Miller, L. R., F. D. Pinkerton, and G. J. Schroepfer, Jr. 1980. 5α-Cholest-8(14)-en-3β-ol-15-one, a potent inhibitor of sterol synthesis, reduces the levels of activity of enzymes involved in the synthesis and reduction of 3-hydroxy-3-methylglutaryl coenzyme A in CHO-Kl cells. *Biochem. Int.* **1:** 223–228.
3. Pinkerton, F. D., A. Izumi, C. M. Anderson, L. R. Miller, A. Kisic, and G. J. Schroepfer, Jr. 1982. 14α-Ethyl-5α-cholest-7-ene-3β,15α-diol, a potent inhibitor of sterol biosynthesis, has two sites of action in cultured mammalian cells. *J. Biol. Chem.* **257:** 1929–1936.
4. Schroepfer, G. J., Jr., D. Monger, A. S. Taylor, J. S. Chamberlain, E. J. Parish, A. Kisic, and A. A. Kandutsch. 1977. Inhibitors of sterol synthesis. Hypocholesterolemic action of dietary 5α-cholest-8(14)-en-3β-ol-15-one in rats and mice. *Biochem. Biophys. Res. Commun.* **78:** 1227–1233.
5. Kisic, A., A. S. Taylor, J. S. Chamberlain, E. J. Parish, and G. J. Schroepfer, Jr. 1978. Inhibitors of sterol synthesis. Further studies of the effect of 5α-cholest-8(14)-en-3β-ol-15-one on serum cholesterol levels of rats. *Fed. Proc.* **37:** 1663 (abstract).
6. Schroepfer, G. J., Jr., E. J. Parish, A. Kisic, E. M. Jackson, C. M. Farley, and G. E. Mott. 1982. 5α-Cholest-8(14)-en-3β-ol-15-one, a potent inhibitor of sterol biosynthesis, lowers serum cholesterol and alters the distribution of cholesterol in lipoproteins of baboons. *Proc. Natl. Acad. Sci. USA.* **79:** 3042–3046.
7. Schroepfer, G. J., Jr., B. C. Sherrill, K-S. Wang, W. K. Wilson, A. Kisic, and T. B. Clarkson. 1984. 5α-Cholest-8(14)-en-3β-ol-15-one lowers serum cholesterol and induces profound changes in the levels of lipoprotein cholesterol and apoproteins in monkeys fed a diet of moderate cholesterol content. *Proc. Natl. Acad. Sci. USA.* **81:** 6861–6865.
8. Monger, D. J., E. J. Parish, and G. J. Schroepfer, Jr. 1980. 15-Oxygenated sterols. Enzymatic conversion of [2,4-^{3}H]-5α-cholest-8(14)-en-3β-ol-15-one to cholesterol in rat liver homogenates. *J. Biol. Chem.* **255:** 11122–11129.
9. Brabson, J. S. 1981. In vivo conversion of 5α-cholest-8(14)-en-3β-ol-15-one to cholesterol in rats. *Fed. Proc.* **40:** 1680 (abstract).
10. Doddrell, D. M., D. T. Pegg, and M. R. Bendall. 1982. Distortionless enhancement of NMR signals by polarization transfer. *J. Magn. Reson.* **48:** 323–327.
11. Bendall, M. R., and D. T. Pegg. 1983. Complete accurate editing of decoupled ^{13}C spectra using DEPT and a quaternary-only sequence. *J. Magn. Reson.* **53:** 272–296.
12. Nakashima, T. T., B. K. John, and R. E. D. McClung. 1984. Selective two-dimensional DEPT heteronuclear shift correlation spectroscopy. *J. Magn. Reson.* **59:** 124–131.
13. Bendall, M. R.. and D. T. Peggs. 1983. ^{1}H-^{13}C Two-dimensional chemical shift correlation spectroscopy using DEPT. *J. Magn. Reson.* **53:** 144–148.
14. Schroepfer, G. J., Jr., E. J. Parish, and A. A. Kandutsch. 1982. Inhibition of sterol biosynthesis by a 15-oxygenated sterol devoided of oxygen functionality at carbon atom 3. *Biochem. Int.* **4:** 263–269.
15. Parish, E. J., T. E. Spike, and G. J. Schroepfer, Jr. 1977. Sterol synthesis. Chemical synthesis of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene, a key intermediate in the synthesis of 15-oxygenated sterols. *Chem. Phys. Lipids.* **18:** 233–239.
16. Schroepfer, G. J., E. J. Parish, and A. A. Kandutsch. 1979. Further studies on the inhibition of sterol biosynthesis in animal cells by 15-oxygenated sterols. *Chem. Phys. Lipids.* **25:** 265–285.
17. Parish, E. J., and G. J. Schroepfer, Jr. 1976. A novel reductive rearrangement of an α,β-unsaturated steroidal epoxide. A new synthesis of 5α-cholest-8(14)-en-3β,15α-diol. *Tetrahedron Lett.* 3775–3778.
18. Parish, E. J., and G. J. Schroepfer, Jr. 1977. Sterol synthesis. A novel reductive rearrangement of an α,β-unsaturated steroidal epoxide: a new chemical synthesis of 5α-cholest-8(14)-en-3β,15α-diol. *Chem. Phys. Lipids.* **18:** 258–266.
19. Parish, E. J., and G. J. Schroepfer, Jr. 1980. Inhibitors of sterol synthesis. Synthesis of [2,4-^{3}H]-5α-cholest-8(14)-ene-3β,7α,15α-triol and [2,4-^{3}H]5α-cholest-8(14)-en-3β-ol-15-one. *J. Labelled Comp. Radiopharm.* **18:** 1429–1436.
20. Midgley, I., and C. Djerassi. 1973. Stereochemical course of the reduction of 5α-cholest-8(14)-en-7-one by metal-ammonia: a ready synthesis of 14β-steroids. *J. Chem. Soc. Perkin I.* 155–161.
21. Tsuda, M., and G. J. Schroepfer, Jr. 1979. Carbon-13 nuclear magnetic resonance studies of C_{27} sterol precursors of cholesterol. *J. Org. Chem.* **44:** 1290–1293.
22. Tsuda, M., and G. J. Schroepfer, Jr. 1981. Inhibitors of sterol biosynthesis. Carbon-13 nuclear magnetic resonance studies of 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one and related compounds. *J. Lipid Res.* **22:** 1188–1197.
23. Schneider, H-J., U. Buchheit, N. Becker, G. Schmidt, and U. Siehl. 1985. ^{1}H NMR analyses, shielding mechanisms, coupling constants, and conformations in steroids bearing halogen, hydroxy, oxo groups, and double bonds. *J. Am. Chem. Soc.* **107:** 7027–7039.
24. Waterhous, D. V., S. Barnes, and D. D. Muccio. 1985. Nuclear magnetic resonance spectroscopy of bile acids. Development of two-dimensional NMR methods for five common hydroxylated bile acids, and their parent bile acid, 5β-cholanoic acid. *J. Lipid Res.* **26:** 1068–1078.
25. Blunt, J. W., and J. B. Stothers. 1977. ^{13}C NMR spectra of steroids—a survey and commentary. *Org. Magn. Reson.* **9:** 439–464.