Chemical synthesis of three 14α-hydroxymethyl cholestenols

Robert A. Pascal, Jr.,[1] Roger Shaw,[2] and George J. Schroepfer, Jr.[3]

Departments of Biochemistry and Chemistry, Rice University, Houston, TX 77001

Abstract Reported herein are chemical syntheses of 14α-hydroxymethyl-5α-cholest-8-en-3β-ol, 14α-hydroxymethyl-5α-cholest-7-en-3β-ol, and 14α-hydroxymethyl-5α-cholest-6-en-3β-ol. These compounds were obtained in pure form after repeated medium-pressure column chromatography of the mixture obtained by treatment of 3β-acetoxy-7α,32-epoxy-14α-methyl-5α-cholestane with pyridine hydrochloride in refluxing acetic anhydride followed by reduction with lithium aluminum hydride. The compounds were characterized by their chromatographic properties and by the results of infrared, optical rotation, nuclear magnetic resonance, and low and high resolution mass spectral studies.—**Pascal, R. A., Jr., R. Shaw, and G. J. Schroepfer, Jr.** Chemical syntheses of three 14α-hydroxymethyl cholestenols. *J. Lipid Res.* 1979. **20:** 570–578.

Supplementary key words 14α-hydroxymethyl sterols · sterols · sterol synthesis

The enzymatic conversion of lanosterol (4α,4β,14α-trimethyl-cholesta-8,24-dien-3β-ol)[4] to cholesterol involves three general events: the removal of the three "extra" methyl groups at carbon atoms 4 and 14 of lanosterol, the reduction of the Δ^{24}-double bond, and the "shift" of the Δ^{8}-nuclear double bond in lanosterol to the Δ^{5}-position in cholesterol. The overall conversion of lanosterol to cholesterol involves a very large number of potential intermediates (1–4). These potential intermediates can show structural variations due to the presence or absence of the Δ^{24}-double bond in the side chain, variations in the order of removal of the extra methyl groups (carbon atoms 30, 31, and 32), variability with respect to the state of oxidation at carbon atom 3 (3-one or 3β-ol), possible hydroxylated intermediates, potential nuclear double bond variants (Δ^{8}, $\Delta^{8(14)}$, Δ^{7}, Δ^{5}, $\Delta^{8,14}$, $\Delta^{7,14}$, $\Delta^{7,9(11)}$, and $\Delta^{5,7}$), and variations due to intermediate oxidation states in the enzymatic removal of a given methyl group. With respect to the latter point, it has generally been considered that the first step in the enzymatic removal of each of the extra methyl groups of lanosterol is an oxidation to give the corresponding hydroxymethyl compound (1–7). For studies relative to the mechanisms involved in the enzymatic removal of the 14α-methyl group of cholesterol precursors and for studies of the regulation of sterol biosynthesis, we have pursued the syntheses of a number of 14α-hydroxymethyl cholestenols. The primary purpose of this communication is to describe the chemical syntheses and properties of 14α-hydroxymethyl-cholest-6-en-3β-ol, 14α-hydroxymethyl-cholest-7-en-3β-ol, and 14α-hydroxymethyl-cholest-8-en-3β-ol. A preliminary account of the syntheses of the former two sterols has been published (8).[5] 14α-Hydroxymethyl-cholest-7-en-3β-ol has been reported to be convertible to cholesterol upon incubation with rat liver homogenate preparations (12).[5] 14α-Hydroxymethyl-cholest-6-en-3β-ol and 14α-hydroxymethyl-cholest-7-en-3β-ol have recently been found to be potent inhibitors of the synthesis of digitonin-precipitable sterols from labeled acetate in L cells (mouse fibroblasts) and in primary cultures of fetal mouse liver cells and to reduce the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in these cells (8).

EXPERIMENTAL PROCEDURES AND RESULTS

General procedures

Melting points were recorded on a Thomas Hoover melting point apparatus or, if the quantity of material available was very small, on a Fisher-Johns melting point apparatus. Infrared (IR) spectra were

Abbreviations: IR, infrared; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance.

[1] Recipient of a Predoctoral Fellowship from the National Science Foundation.

[2] Present address, Department of Biochemistry, St. Louis University School of Medicine.

[3] To whom inquiries should be directed.

[4] The configuration of the hydrogen at carbon atom 5 in the various sterols mentioned in this paper is α. The designation of the configuration as 5α has been omitted throughout the text to conserve space.

[5] These results have also been presented at three symposia (9–11).

Downloaded from www.jlr.org by guest, on June 19, 2012

recorded on a Beckman IR-9 spectrometer using KBr pellets. Nuclear magnetic resonance (NMR) spectra were determined on $CDCl_3$ solutions of the sterols on a Perkin-Elmer Model HR-12 spectrometer at 60 MHz or on a Varian EM-390 spectrometer at 90 MHz using tetramethylsilane (TMS) as an internal standard. Peaks are reported as ppm (δ) downfield from the TMS standard. Low-resolution mass spectra (MS) were recorded on a CEC Model 110-B spectrometer or on an LKB-9000S spectrometer. High-resolution mass spectral measurements were made on a Varian CH-5 spectrometer (courtesy of Dr. C. C. Sweeley). Optical rotations were measured on a JASCO DIP-4 digital polarimeter on chloroform solutions of the sterols. Gas–liquid chromatographic (GLC) analyses were made using a Hewlett-Packard Model 402 unit equipped with dual flame ionization detectors. All-glass columns (6 ft × ⅛ in) packed with either 1% QF-1, 3% OV-1, or 3% OV-17 on Gas Chrom Q (100–120 mesh) were used. The preparation of trimethylsilyl derivatives of the sterols was carried out as described previously (13). Analyses by combined GLC–MS were made using a 3% OV-1 column and an LKB-9000S spectrometer. Operating conditions for the mass spectrometer were as described previously (14). Analytical thin-layer chromatographic (TLC) analyses were carried out on precoated silica gel G or silica gel GF plates (7.5 × 2.0 cm or 20 × 10 cm; Analtech, Newark, DE). Components on the plates were detected after spraying with molybdic acid (15). Preparative TLC was carried out on precoated plates of silica gel $PF_{254+366}$. Components on preparative plates were detected as fluorescent bands under ultraviolet light after spraying with a solution of Rhodamine 6G in acetone.

Materials

Dioxane and tetrahydrofuran were refluxed over sodium for several days under dry nitrogen (purified by passage through Fehling's solution and Drierite) and then distilled under the same inert atmosphere into a reaction flask as needed. Hexamethylphosphoramide was distilled from potassium hydroxide. 2-Propylamine and *n*-butyl bromide were distilled prior to use. Potassium *t*-butoxide was purified by sublimation and stored in a desiccator. Sodium hydride (50% in oil) was washed thoroughly with ether immediately prior to use. Anhydrous ethylamine was prepared by treating 70% ethylamine in water with ½ its weight of sodium hydroxide pellets. The ethylamine was distilled off and stored over molecular sieves (Linde type 3A). Prior to use, the ethylamine was dried overnight over anhydrous magnesium sulfate. Chloromethyl benzyl ether was prepared as described by Rieche and Gross (16) and phenyl formate was prepared according to van Es and Stevens (17). Lithium diisopropylamide was prepared according to House et al. (18) except that *n*-butyl lithium was used in place of methyl lithium. Tritylithium was prepared according to Tomboulian and Stehower (19). 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one (I) (see **Fig. 1**) was prepared by a modification (20) of the method described by Knight, Klein, and Szczepanik (21). 5α-Cholest-8(14)-en-3β-ol-15-one (II; single component on TLC; solvent system, ethyl acetate–benzene 1:2) was prepared from the corresponding 3β-benzoate ester by a slight modification of a procedure described previously (22). 3β-Benzoyloxy-14α-methyl-cholest-7-en-15-one (III) was prepared by a modification (23) of the method of Knight et al. (21) and Woodward et al. (24).

3β-Tetrahydropyranyloxy-cholest-8(14)-en-15-one (IV)

3β-Hydroxy-cholest-8(14)-en-15-one (0.42 g) was swirled in a mixture of concentrated hydrochloric acid (~10 μl) and dihydropyran (2 ml) until complete solution occurred. After standing at room temperature for 24 hr the mixture was evaporated to dryness and the residue was dissolved in ether. The resulting ether solution was washed successively with a saturated solution of sodium bicarbonate and water, dried over anhydrous magnesium sulfate, and evaporated to dryness. Crystallization from 90% ethanol gave colorless needles (0.458 g; 90% yield) which showed, on TLC analysis (solvent, ethyl acetate–benzene 1:19), two spots of equal intensity (R_f 0.36 and 0.44); NMR, 0.70 (s, 3H, C-18-CH_3), 0.96 (s, 3H, C-19-CH_3), 3.59 (m, 3H, C-3-H and 6′-H′s of pyranyl residue), 4.10 (m, 1H, C-7-H), and 4.69 (m, 1H, 2′-H of pyranyl residue); MS, 484 (M; 35%), 400 (M − pyranyl group; 28%), 382 (M − pyranyl group − H_2O; 4%), 367 (M − pyranyl group − H_2O − CH_3; 8%), and 55 (100%).

Attempts at direct introduction of benzyloxymethyl and formyl groups at carbon atom 14

Attempts at alkylation of 3β-benzoyloxy-cholest-8(14)-en-15-one with chloromethyl benzyl ether in dioxane using either sodium hydride, tritylithium, or lithium diisopropylamide as base under a variety of conditions were unsuccessful. An attempt at formylation of 3β-benzoyloxy-cholest-8(14)-en-15-one in tetrahydrofuran with phenyl formate using potassium *t*-butoxide as base was similarly unsuccessful, as was an attempt to alkylate 3β-tetrahydropyranyloxy-cholest-8(14)-en-15-one with chloromethyl benzyl ether using *n*-butyl magnesium bromide as base.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

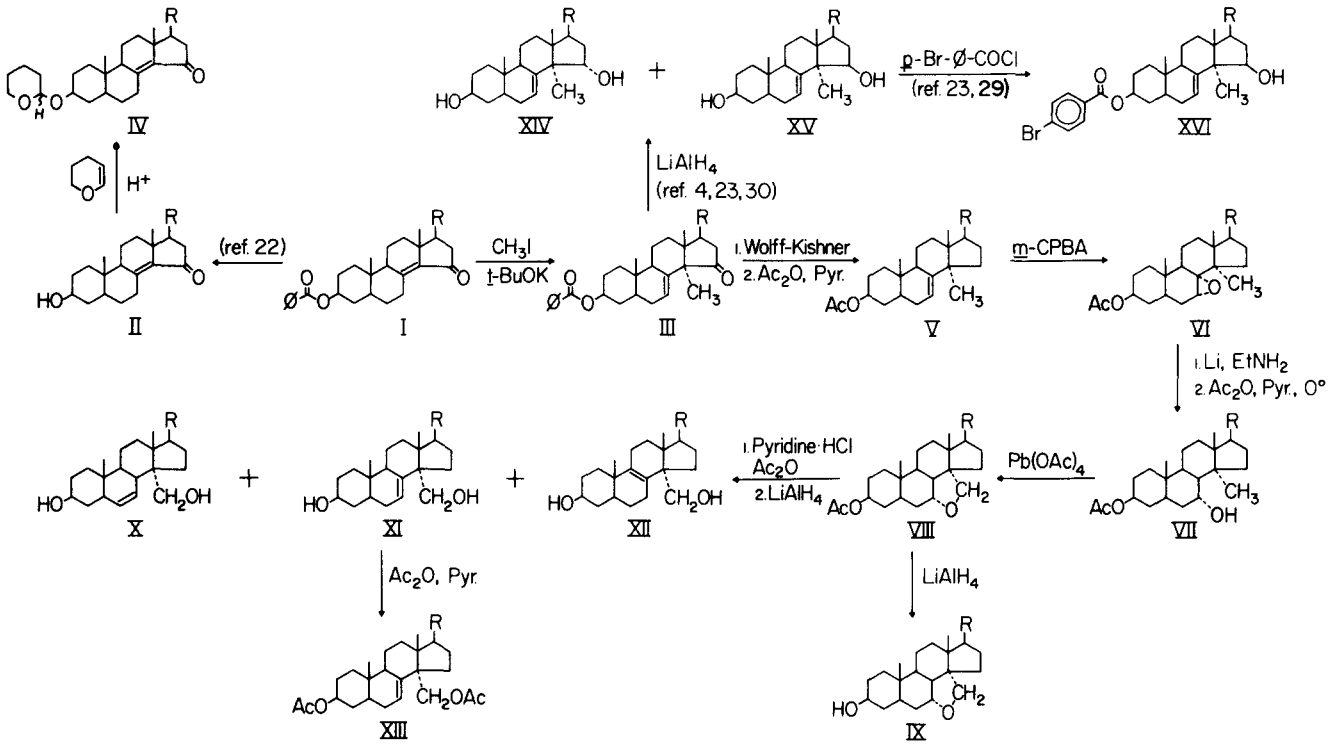

Fig. 1. Synthesis and stereochemical interrelationships of 14α-hydroxymethyl sterols.

3β-Acetoxy-14α-methyl-cholest-7-ene (V)

A modification[6] of the procedure of Knight et al. (21) was used to prepare 3β-acetoxy-14α-methyl-cholest-7-ene in 96% yield, mp 95.5–96.5°C (lit. 95–96°C (24) and 96–97°C (21)); IR, ν_{max} 2960, 1730, 1474, 1385, 1249, and 1029 cm^{-1}; NMR, 0.65 (s, 3H, C-18-CH_3), 0.82 (s, 3H, C-19-CH_3), 0.97 (s, 3H, C-32-CH_3), 1.99 (s, 3H, methyl of acetoxy function), 4.69 (m, 1H, C-3-H), and 5.15 (m, 1H, C-7-H); MS, 442 (M; 20%), 427 (M − CH_3; 100%), 367 (M − CH_3 − CH_3COOH; 71%), 242 (20%), 227 (30%), and 201 (18%). The compound showed a single component on GLC (3% OV-17; 270°C).

3β-Acetoxy-7α,8α-epoxy-14α-methyl-cholestane (VI)

A modification of the procedure of Knight, Belletire, and G. R. Pettit (25) was used to prepare 3β-acetoxy-7α,8α-epoxy-14α-methyl-cholestane in 92% yield, mp 98–100°C (lit. 101–102°C (25)); IR, ν_{max} 2965, 1739, 1469, 1363, 1247, and 1029 cm^{-1}; NMR, 0.81 (s, 3H, C-18-CH_3), 0.86 (s, 3H, C-19-CH_3), 1.05 (s, 3H, C-32-CH_3) (lit., 1.06 (C-32-CH_3) (25)), 1.99 (s, ~3H, methyl of acetoxy function), 3.35 (m, 1H, C-7-H) (lit., 3.3 (7-H) (25)), and 4.67 (m, 1H, C-3-H); MS, 458 (M; 10%), 443 (M − CH_3; 5%), 440 (M − H_2O; 6%), 365 (M − CH_3 − H_2O − CH_3COOH; 4%), 304 (5%), and 120 (100%). The purified compound showed a single component on TLC analysis (solvent, ethyl acetate–benzene 1:19) with an R_f value of 0.35.

3β-Acetoxy-14α-methyl-cholestan-7α-ol (VII)

3β-Acetoxy-14α-methyl-cholestan-7α-ol was prepared in 62.5% overall yield from 3β-acetoxy-7α,8α-epoxy-14α-methyl-cholestane by a modification of the two-step procedure of Knight et al. (25) The product melted at 159–160°C (lit. 156–158°C (25)); IR, ν_{max} 3495, 2955, 1718, 1469, 1376, 1277, and 1029 cm^{-1}; MS, 460 (M; 5%), 442 (M − H_2O; 16%), 427 (M − H_2O − CH_3; 37%), 367 (M − H_2O − CH_3 − CH_3COOH; 30%), 305 (55%), 302 (100%), 274 (31%), 236 (45%), 227 (35%), 220 (85%), and 202 (32%); NMR, 0.75 (s, 3H, C-18-CH_3), 0.89 (s, 3H, C-19-CH_3), 1.09 (s, 3H, C-32-CH_3), 1.99 (s, 3H, methyl of acetoxy function), 3.98 (m, 1H, C-7-H), and 4.72 (m, 1H, C-3-H). Analysis by TLC (silica gel, 10% ether in benzene) showed a single component (R_f 0.41).

[6] In the past, in our laboratory and in others, this Wolff–Kishner reduction has been performed with only mixed success. However, overall yields consistently in excess of 95% can be obtained by scrupulous exclusion of water from the reaction mixture. We should be pleased to supply any inquirers with a copy of our procedure and a diagram of the apparatus used for carrying out this reaction.

Downloaded from www.jlr.org by guest, on June 19, 2012

3β-Acetoxy-7α,32-epoxy-14α-methyl-cholestane (VIII)

A slight modification of the procedure of Knight et al. (25) was used to prepare 3β-acetoxy-7α,32-epoxy-14α-methyl-cholestane in 49% yield, mp 136–137°C (lit. 133–134°C (25)); IR, ν_{max} 2955, 1743, 1473, 1372, 1241, and 1029 cm^{-1}; MS, 458 (M; 3%), 443 (M − CH_3; 3%) 427 (82%), 413 (12%), 367 (34%), 353 (11%), 345 (M − side chain; 12%), 313 (100%), 255 (14%), 253 (17%), 229 (12%), and 213 (14%); NMR, 0.75 (s, 3H, C-18-CH_3), 0.88 (s, 3H, C-19-CH_3), 1.98 (s, 3H, methyl of acetoxy function), 3.35 (d, 1H, C-32-H; J = 7.5 Hz), 3.99 (d, 1H, C-32-H; J = 7.5 Hz), 4.06 (m, 1H, C-7-H), and 4.69 (m, 1H, C-3-H). The compound showed a single component on TLC (solvent, 5% ether in benzene; R_f 0.25).

7α,32-Epoxy-14α-methyl-cholestan-3β-ol (IX)

To 3β-acetoxy-7α,32-epoxy-14α-methyl-cholestane (11 mg) in ether (10 ml) was added lithium aluminum hydride (70 mg). After standing 1 hr at room temperature, ice, water, and 4 N HCl were successively added to decompose the excess hydride. The resulting mixture was extracted with ether and the combined extracts were evaporated to dryness under nitrogen to give a white crystalline residue (~10 mg) which showed one component on TLC (solvent, benzene–ether 3:2). This material was dissolved in $CDCl_3$ and the NMR spectrum was recorded: 0.74 (s, 3H, C-18-CH_3), 0.88 (s, 3H, C-19-CH_3), 3.36 (d, 1H, C-32-H; J = 7.5 Hz), 3.60 (m, 1H, C-3-H), 4.00 (d, 1H, C-32-H; J = 7.5 Hz), and 4.07 (m, 1H, C-7β-H). The residue obtained upon evaporation of the solvent was recrystallized from acetone–water to give 7α,32-epoxy-14α-methyl-cholestan-3β-ol (5.1 mg) as crystals which, upon heating to 116–119°C, first softened, became transparent, and then solidified. Upon further heating the compound melted at 140–142°C; IR, ν_{max} 3370, 2945, 1472, 1381, 1052, 1023, 981, and 820 cm^{-1}; MS, 416 (M; 3%), 401 (M − CH_3; 3%), 386 (M − CH_2O; 53%), 385 (M − CH_2OH; 90%), 371 (M − CH_2O − CH_3; 17%), 367 (M − CH_2OH − H_2O; 18%), 303 (M − side chain; 13%), 273 (16%), and 271 (100%); high resolution MS, 416.3640 (calc. for $C_{28}H_{49}O_2$: 416.3654). Analysis by TLC (solvent, 50% ether in benzene) showed a single component (R_f 0.20). Analysis by GLC of the trimethylsilyl ether derivative (3% OV-17) indicated the purity to be in excess of 99%. At 260°C the compound had a retention time (relative to cholestane) of 4.93. The trimethylsilyl ether derivative showed the following prominent ions on analysis by GLC–MS: 488 (M; 4%), 473 (M − CH_3; 25%), 458 (M − CH_2O; 76%), 457 (M − CH_2OH; 100%), 443 (M − CH_2O − CH_3; 10%), 367 (M − CH_2OH − trimethylsilanol; 79%), 353 (M − CH_2O − CH_3 − trimethylsilanol; 24%), and 343 (94%).

14α-Hydroxymethyl-cholest-6-en-3β-ol (X), 14α-hydroxymethyl-cholest-7-en-3β-ol (XI), and 14α-hydroxymethyl-cholest-8-en-3β-ol (XII)

3β-Acetoxy-7α,32-epoxy-14α-methyl-cholestane (475 mg; 1.04 mmol) was heated under reflux with pyridine hydrochloride (900 mg) and acetic anhydride (20 ml) for 5 hr under nitrogen. After cooling to room temperature, water (20 ml) was added. After standing for 2 hr at room temperature, the mixture was diluted with water and extracted three times with ether (100-ml portions). The combined extracts were washed successively with 2.5% HCl, water, a saturated solution of sodium carbonate, and water, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. To the resulting white solid in ether was added lithium aluminum hydride (600 mg). After standing 20 min at room temperature, ice and a 2.5% HCl solution were successively added and the resulting mixture was extracted three times with ether (100-ml portions). The combined extracts were successively washed with water, a saturated solution of sodium bicarbonate, and water, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. Analysis by TLC (solvent, 50% U.S.P. ether in benzene) showed three components (R_f values of 0.32, 0.27, and 0.23). The mixture was subjected to medium-pressure silica gel (Woelm; 0.032–0.063 mm) column (100 × 1.5 cm) chromatography using a mixture of ether and benzene (2:3) as the eluting solvent at a flow rate of 3.1 ml per min. At fraction 68 the eluting solvent was changed to a mixture of ether and benzene (1:1). Fractions of 12.4 ml in volume were collected.

The contents of fractions 75–98 were pooled and, after evaporation of the solvent, recrystallized from acetone to give 14α-hydroxymethyl-cholest-6-en-3β-ol (58 mg; 13% yield). Examination by TLC indicated a trace of contamination by material of R_f 0.27. The product was subjected to further purification on a silica gel (60–200 mesh) column (45 × 1.0 cm) using benzene–ether 3:2 as the eluting solvent. The purified product, crystallized from acetone, melted at 204–205°C, IR, ν_{max} 3320, 2920, 1460, 1373, 1050, 1029, and 752 cm^{-1}; NMR, 0.82 (s, 3H, C-19-CH_3), 0.92 (s, 3H, C-18-CH_3), 3.46 (d, 1H, C-32-H; J = 12 Hz), 3.65 (m, 1H, C-3-H), 4.19 (d, 1H, C-32-H; J = 12 Hz), 5.35 (d, 1H, C-7-H; J = 10 Hz), and 5.76 (d, 1H, C-6-H; J = 10 Hz); $[\alpha]_D$ −42° (*c*. 0.06);

Downloaded from www.jlr.org by guest, on June 19, 2012

MS, 416 (M; 1%), 398 (M − H_2O; 41%), 385 (M − CH_2OH; 100%), 367 (M − H_2O − CH_2OH; 57%), 365 (13%), 259 (21%), 245 (10%), 231 (12%), 230 (16%), 227 (15%), 220 (11%), 213 (14%), and 207 (12%); high resolution MS on ion at *m/e* 385, 385.3459 (calc. for $C_{27}H_{45}O$: 385.3470). The compound showed a single component on TLC (solvent, ether–benzene 1:1; R_f 0.25). The *bis*-trimethylsilyl ether derivative showed a single compound on GLC (3% OV-1; 255°C) with a retention time (relative to cholestane) of 2.73. The trimethylsilyl derivative showed the following prominent ions on GLC–MS analysis: 560 (M; 0.8%), 545 (M − CH_3; 4%), 470 (M − trimethylsilanol; 74%), 457 (M − $CH_2OSi(CH_3)$; 38%), 380 M − trimethylsilanol − trimethylsilanol; 45%), and 367 (M − $CH_2OSi(CH_3)_3$ − trimethylsilanol; 100%).

The contents of fractions 99–118 and 119–188 (from the initial medium-pressure column) were pooled separately and each was further purified by three additional medium-pressure runs. Ultimately, pure material corresponding to 14α-hydroxymethyl-cholest-8-en-3β-ol and 14α-hydroxymethyl-cholest-7-en-3β-ol was obtained.

The former material was recrystallized from acetone to give 14α-hydroxymethyl-cholest-8-en-3β-ol (68 mg; 16% yield) melting at 198–200°C; IR, ν_{max} 3310, 2915, 1462, 1369, 1048, and 1017 cm^{-1}; NMR, 0.70 (s, 3H, C-18-CH_3), 0.98 (s, 3H, C-19-CH_3), 3.19 (d, 1H, C-32-H; J = 11 Hz), ~3.60 (m, 1H, C-3-H), and 3.62 (d, 1H, C-32-H; J = 11 Hz); $[\alpha]_D$ +77° (*c*. 0.39); MS, 416 (M; 0.2%), 398 (M − H_2O; 6%), 385 (M − CH_2OH; 100%), 367 (M − H_2O − CH_2OH; 10%), 285 (11%), 273 (10%), 231 (14%), and 213 (12%), high resolution MS on ion at *m/e* 398, 398.3420 (calc. for $C_{27}H_{47}O_2$: 398.3458). The compound showed a single component on TLC (solvent, ether–benzene 1:1; R_f 0.20). The trimethylsilyl ether derivative showed a single component on analysis by GLC (3% OV-1, 255°C). The trimethylsilyl derivative showed the following prominent ions on GLC–MS analysis: 560 (M; 0.2%), 545 (M − CH_3; 8%), 470 (M − trimethylsilanol; 3%), 458 (M − $CHOSi(CH_3)_3$; 80%), 457 (M − $CH_2OSi(CH_3)_3$; 100%), and 367 (M − $CH_2OSi(CH_3)_3$ − trimethylsilanol; 94%).

The latter material from the repeated column chromatography was recrystallized from acetone to yield 14α-hydroxymethyl-cholest-7-en-3β-ol (185 mg; 43% yield) melting at 185–186°C (no change in mp upon recrystallization from methanol); IR, ν_{max} 3310, 2910, 1463, 1373, and 1040 cm^{-1}; NMR, 0.73 (s, 3H, C-18-CH_3), 0.83 (s, 3H, C-19-CH_3), 3.22 (dd, 1H, C-32-H; J = 10 Hz, 10 Hz; collapsed to d, 1H, J = 10 Hz on exchange with D_2O), 3.56 (m, 1H, C-3-H), 3.66 (d, 1H, C-32-H; J = 10 Hz), and 5.30 (m, 1H, C-7-H); $[\alpha]_D$ +18.6° (*c*. 0.42); MS, 416 (M; 2%), 401 (M − CH_3; 2%), 398 (M − H_2O; 1%), 385 (M − CH_2OH; 100%), and 367 (M − CH_2OH − H_2O; 44%); high resolution MS on ion at *m/e* 385, 385.3443 (calc. for $C_{27}H_{45}O$: 385.3470). The compound showed a single component on TLC (silica gel G; ether–benzene 1:1; R_f 0.17) and on GLC (3% OV-1; 255°C) of the trimethylsilyl derivative with a retention time (relative to cholestane) of 2.76. The trimethylsilyl derivative showed the following prominent ions on GLC–MS analysis: 560 (M; 0.5%), 545 (M − CH_3; 9%), 470 (M − trimethylsilanol; 24%), 458 (M − $CHOSi(CH_3)_3$; 92%), 457 (M − $CH_2OSi(CH_3)_3$; 100%), and 367 (M − $CH_2OSi(CH_3)_3$; 94%).

3β-Acetoxy-14α-acetoxymethyl-cholest-7-ene (XIII)

14α-Hydroxymethyl-5α-cholest-7-en-3β-ol (20 mg; 0.048 mmol) was treated with acetic anhydride (1.5 ml) in pyridine (1.5 ml) for 3 hr at 50°C. The residue obtained upon evaporation of the solvent under reduced pressure was dissolved in benzene (2 ml) and subjected to chromatography on an activated silicic acid column (0.7 × 20 cm; Unisil; Clarkson Chemical Company) using 2.5% ether in benzene as the eluting solvent. Fractions 6 ml in volume were collected. The contents of fractions 6–11 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from acetone–water to give 3β-acetoxy-14α-acetoxymethyl-cholest-7-ene (13 mg; 54% yield) melting at 103.5–104.5°C (lit. 103–105°C (25)); $[\alpha]_D$ +18° (*c*. 0.2) (lit. +18.7° (25)); IR, ν_{max} 2955, 1743, 1470, 1380, 1250, and 1038 cm^{-1}; MS, 500 (M; 0.2%), 440 (M − CH_3COOH; 10%), 427 (M − CH_2OOCCH_3; 100%), 380 (M − CH_3COOH − CH_3COOH; 3%), 367 (M − CH_3COOH − CH_2OOCCH_3; 50%), and 327 (M − CH_3COOH − side chain; 32%); NMR, 0.71 (s, 3H, C-18-CH_3), 0.82 (s, 3H, C-19-CH_3), 1.95 (s, 3H, methyl of acetoxy function), 1.99 (s, 3H, methyl of acetoxy function), 3.71 (d, 1H, C-32-H; J = 11 Hz), 4.56 (d, 1H, C-32-H; J = 11 Hz), 4.65 (m, 1H, C-3-H), and 5.14 (m, 1H, C-7-H). The compound showed a single component on TLC (solvent, 5% ether in benzene; R_f = 0.42). Analysis by GLC (3% OV-17, 280°C) indicated one major component (98.6%) with a retention time (relative to cholestane) of 7.2.

DISCUSSION

Our initial efforts towards the syntheses of 14α-hydroxymethyl-substituted cholestenols were based upon attempts to alkylate 3β-benzoyloxy-cholest-8(14)-en-15-one (I) or 3β-tetrahydropyranyloxy-cholest-8(14)-en-15-one (IV) with chloromethyl benzyl

Downloaded from www.jlr.org by guest, on June 19, 2012

ether in the presence of a number of bases under a variety of conditions. These attempts were unsuccessful, as were attempts at formylation of I with phenyl formate. We thereupon directed our efforts towards a method, described in preliminary form by Fried et al.. (26, 27), for the synthesis of 14α-hydroxymethyl-4,4-dimethyl-cholest-7-en-3β-ol. This approach is outlined in Fig. 1 for the cases of the 14α-hydroxymethyl cholestenols. A key intermediate in this approach is a 7α,32-epoxide. Fried, Brown, and Borkenhagen (26) reported that formation of this tetrahydrofuran ring system could be effected by treatment of the 14α-methyl-7α-hydroxysterol derivative with lead tetraacetate and that acetolytic cleavage of the tetrahydrofuran ring system to give the corresponding Δ^7-32-acetoxy derivative could be effected by treatment of the epoxide with acetic anhydride and pyridine hydrochloride. Knight et al. (25), using this general approach, described the preparation of 3β-acetoxy-7α,32-epoxy-cholestane (VIII). These workers reported that treatment of the epoxide (VIII) with acetic anhydride and pyridine hydrochloride gave a complex mixture from which 3β-acetoxy-14α-acetoxymethyl-cholest-7-ene (XIII) was isolated in low yield. The latter compound was characterized by its melting point, optical rotation, and by the results of NMR, mass spectral, and elemental analyses. Akhtar et al. (28) have recently reported that treatment of 3β-acetoxy-7α,32-epoxy-5α-lanostane with pyridine hydrochloride in refluxing acetic anhydride gave, after base hydrolysis, a mixture of dihydroxysterols. These workers reported isolation of 5α-lanost-6-en-3β,32-diol, 5α-lanost-7-en-3β,32-diol, and 5α-lanost-8-en-3β,32-diol upon repeated TLC. The compounds in question were characterized by their melting points and optical rotations and by the results of NMR and, in the case of the latter two sterols, by low-resolution MS analyses.

The basic synthetic scheme used in the present study is outlined in Fig. 1. The ultimate starting material for this synthesis is the $\Delta^{8(14)}$-15-ketosteryl benzoate (I). 3β-Benzoyloxy-14α-methyl-cholest-7-ene (III) was prepared from I by a modification (23) of the method described by Knight et al. (21) and Woodward et al. (24). That the alkylation proceeds to yield the 14α-methyl compound (i.e., *trans* C-D ring junction) has been unequivocally established by x-ray crystallographic analysis (23, 29) of the 3β-*p*-bromobenzoate derivative (XVI) of 14α-methyl-cholest-7-en-3β,15β-diol (XV), one of the two epimeric (at C-15) diols (XIV and XV) obtained upon lithium aluminum hydride reduction of the corresponding ketone (III). Pure 3β-acetoxy-14α-methyl-cholest-7-ene (V) was obtained from III in very high yield (96%) by a modification of the procedure of Knight et al. (21). Treatment of the 14α-methyl-Δ^7-steryl acetate with *m*-chloroperbenzoic acid gave the 7α,8α-epoxide (VI) whose physical and spectral properties were in good agreement with those reported by Knight et al. (25). Reduction of the 7α,8α-epoxide (VI) with lithium and ethylamine gave 3β-acetoxy-14α-methyl-cholestan-7α-ol (VII) which was obtained in pure form by a modification of the procedure of Knight et al. (25). Treatment of VII with lead tetraacetate, by a modification of the conditions described by Knight et al. (25), gave pure 3β-acetoxy-7α,32-epoxy-14α-methyl-cholestane (VIII) which was fully characterized as such and, after reduction with lithium aluminum hydride, as the free sterol (IX). Treatment of VIII with pyridine hydrochloride in refluxing acetic anhydride gave, after standard processing, a crude product which was reduced with lithium aluminum hydride in ether. The resulting product was shown by TLC to be a mixture of three components. Repeated medium-pressure silica gel column chromatography gave 14α-hydroxymethyl-cholest-6-en-3β-ol (X), 14α-hydroxymethyl-cholest-7-en-3β-ol (XI), and 14α-hydroxymethyl-cholest-8-en-3β-ol (XII) in yields of 13%, 43%, and 16%, respectively. Each of the three sterols was characterized by its melting point and optical rotation, and by the results of IR, NMR, and low and high resolution mass spectral analyses. Each of the sterols was shown to possess two hydroxyl functions by the results of GLC–MS analysis of its trimethylsilyl ether derivative.

The locations of the nuclear double bonds were established by the results of the NMR and optical rotation studies. The presence of a Δ^6-nuclear double bond in X was indicated by the presence, in the NMR spectrum, of two olefinic proton resonances which formed an AX spin system (J_{AX} = 10 Hz). The presence of a Δ^7-double bond in XI was indicated by the single olefinic proton resonance in its NMR spectrum, and the presence of a Δ^8-double bond in XII was suggested by the absence of any olefinic proton resonances in its NMR spectrum. These assignments were further supported by the positions of the C-18 and C-19 methyl resonances in the NMR spectra of these compounds (**Table 1**). 14α-Hydroxymethyl-cholest-7-en-3-one was prepared by oxidation of the 3β-hydroxyl function of XI using cholesterol oxidase.[7] Comparison of the NMR spectra of XI and the 3-ketosterol in the light of Zurcher's tables (31) allowed unambiguous assignment of their angular methyl group resonances. Then, by using

[7] G. J. Schroepfer, E. J. Parish, R. A. Pascal, Jr., and A. A. Kandutsch. Manuscript in preparation.

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 1. C-18 and C-19 methyl group resonances for 14α-hydroxymethyl sterols

Compound	C-18		C-19	
	$\delta_{obs.}$	$\delta_{calc.}$	$\delta_{obs.}$	$\delta_{calc.}$
14α-Hydroxymethyl-cholest-7-en-3β-ol (XI)	0.70		0.835	
14α-Hydroxymethyl-cholest-7-en-3-one	0.75	0.76	1.05	1.04
14α-Hydroxymethyl-cholest-6-en-3β-ol (X)	0.92	0.90	0.82	0.82
14α-Hydroxymethyl-cholest-8-en-3β-ol (XII)	0.70	0.76	0.98	0.97
4,4-Dimethyl-14α-hydroxy-methyl-cholest-7-en-3β-ol (28)	0.72		0.91	
4,4-Dimethyl-14α-hydroxy-methyl-cholest-8-en-3β-ol (28)	0.71	0.76	1.04	1.03

Zurcher's tables and the angular methyl group resonances of XI as a base, it was possible to calculate the expected C-18 and C-19 methyl group resonances for the remaining 14α-hydroxymethyl sterols. The data presented in Table 1 show that the calculated and observed values for these resonances were in close agreement, thus supporting the nuclear double bond assignments. The only significant discrepancy observed was that between the calculated (δ 0.76) and observed (δ 0.70) values for the C-18 methyl group resonance of XII. The data of Akhtar et al. (28) show a similar discrepancy between the calculated (δ 0.76) and observed (δ 0.71) values for the C-18 methyl group resonance of 4,4-dimethyl-14α-hydroxymethyl-cholest-8-en-3β-ol (Table 1). This small, but significant, discrepancy probably reflects the limitations of applying the purely empirical Zurcher tables to 14α-hydroxymethyl sterols, which these tables do not specifically treat.

The observed specific optical rotations for X, XI, and XII were also consistent with their structural assignments. The reported specific rotations of cholest-6-ene, cholest-7-ene, and cholest-8-ene are −88°, +12°, and +56°, respectively (32). Reported specific rotations for cholest-6-en-3β-ol, cholest-7-en-3β-ol, and cholest-8-en-3β-ol are −97.5° (33), +3.4° (33), and +50° (34), respectively. The specific rotations of the 14α-hydroxymethyl sterols X, XI, and XII were −42°, +18.6°, and +77°, respectively. These findings suggest that the 14α-hydroxymethyl group causes a significant positive shift in the rotation in these molecules. The general trend of the rotations of X, XI, and XII is clearly compatible with the presence of Δ^6-, Δ^7-, and Δ^8-double bonds, respectively, in their structures.

The Δ^7-sterol (XI) was also converted to its 3β,32-diacetate derivative (XIII), which had essentially the same melting point and specific optical rotation as reported by Knight et al. (25).

The chromatographic mobilities of the three hydroxymethyl sterols and the epoxide IX were characterized in several systems. **Table 2** presents the results of TLC analyses of these sterols on silica gel and on alumina–silver nitrate (35) plates. It is important to note that the R_f of the epoxide IX is very similar to that of XII in the first of these systems. Also, the very low R_f for X on argentation chromatography is also compatible with the presence of a Δ^6-nuclear double bond in its structure. **Table 3** summarizes the behavior of the trimethylsilyl derivatives of these sterols upon GLC analyses.

The preparation of 14α-hydroxymethyl-cholest-7-en-3β-ol by another route has recently been reported by Anastasia et al. (36). While the value of the specific optical rotation and the NMR data reported by the latter workers are in close agreement with those reported herein, their reported melting point (124–125°C) is considerably lower than that (185–186°C) observed in the present study. The reason for this discrepancy is not readily apparent. The stereochemical orientation of carbon atom 32 of 3β-acetoxy-7α,32-epoxy-14α-methyl-cholestane, and hence of the same carbon in compounds X, XI, and XII, rests on a body of evidence, the most compelling of which is the unequivocal determination of the stereochemical orientation of a derivative (XVI) of 14α-methyl-cholest-7-en-3β,15β-diol (XV) which was prepared from 3β-benzoyloxy-14α-methyl-cholest-7-en-15-one (XV) (23, 29). As noted in Fig. 1, the latter compound can be considered as the starting material for the synthesis of the 7α,32-epoxide.

As noted previously, 14α-hydroxymethyl-cholest-7-en-3β-ol has been shown to be convertible to cholesterol upon incubation with rat liver homogenate

TABLE 2. R_f values for 14α-hydroxymethyl sterols on thin-layer chromatography

Compound	Silica gel G (Solvent ether:benzene 1:1)	Alumina–Silver Nitrate (Solvent chloroform:acetone 9:1)
14α-Hydroxymethyl-cholest-6-en-3β-ol (X)	0.25	0.22
14α-Hydroxymethyl-cholest-7-en-3β-ol (XI)	0.17	0.56
14α-Hydroxymethyl-cholest-8-en-3β-ol (XII)	0.20	0.63
7α,32-Epoxy-14α-methyl-cholestan-3β-ol (IX)	0.20	0.71

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 3. Retention times (relative to cholestane) of trimethylsilyl derivatives of 14α-hydroxymethyl sterols on gas–liquid chromatography

Compound	Column	
	3% OV-1 (255°C)	3% OV-17 (245°C)
14α-Hydroxymethyl-cholest-6-en-3β-ol (X)	2.73	2.56
14α-Hydroxymethyl-cholest-7-en-3β-ol (XI)	2.76	2.64
14α-Hydroxymethyl-cholest-8-en-3β-ol (XII)	2.44	2.32
7α,32-Epoxy-14α-methyl-cholestan-3β-ol (IX)		5.66

preparations (12).[5] Moreover, this compound and 14α-hydroxymethyl-cholest-6-en-3β-ol have recently been shown to be potent inhibitors of sterol synthesis in L cells and in primary cultures of fetal mouse liver cells and to reduce the levels of HMG-CoA reductase in these cells (8). Further studies of the metabolism and biological effects of the 14α-hydroxymethyl sterols are in progress.

This research was supported by grants from the National Institutes of Health (HL-15376) and the Robert A. Welch Foundation (C-583).

Manuscript received 22 August 1978; accepted 11 December 1978.

REFERENCES

1. Bloch, K. 1965. The biological synthesis of cholesterol. *Science.* **150:** 19–28.
2. Clayton, R. B. 1965. Biosynthesis of sterols, steroids, and terpenoids. Part I. Biogenesis of cholesterol and the fundamental steps in terpenoid biosynthesis. *Quart. Rev.* **19:** 168–200.
3. Frantz, I. D., Jr., and G. J. Schroepfer, Jr. 1967. Sterol biosynthesis. *Ann. Rev. Biochem.* **36,** 691–726.
4. Schroepfer, G. J., Jr., B. N. Lutsky, J. A. Martin, S. Huntoon, B. Fourcans, W-H. Lee, and J. Vermilion. 1972. Recent investigations on the nature of sterol intermediates in the biosynthesis of cholesterol. *Proc. Roy. Soc. London B* **180:** 125–146.
5. Gaylor, J. L., and C. V. Delwiche. 1973. Investigation of the multienzymic system of microsomal cholesterol biosynthesis. *Ann. N.Y. Acad. Sci.* **212:** 122–138.
6. Gibbons, G. F., K. A. Mitropoulos, and C. R. Pullinger. 1976. Lanosterol 14α-demethylase. The metabolism of some potential intermediates by cell-free systems from rat liver. *Biochem. Biophys. Res. Commun.* **69:** 781–789.
7. Akhtar, M., K. Alexander, R. B. Boar, J. F. McGhie, and D. H. R. Barton. 1978. Chemical and enzymic studies on the characterization of intermediates during the removal of the 14α-methyl group in cholesterol biosynthesis. *Biochem. J.* **169:** 449–463.
8. Schroepfer, G. J., Jr., R. A. Pascal, Jr., R. Shaw, and A. A. Kandutsch. 1978. Inhibition of sterol biosynthesis by 14α-hydroxymethyl sterols. *Biochem. Biophys. Res. Commun.* **83:** 1024–1031.
9. Schroepfer, G. J., Jr. 1974. Recent studies on the biosynthesis of cholesterol. Abstracts, 17th International Conference on the Biochemistry of Lipids (Milan, Italy) p. 28.
10. Schroepfer, G. J., Jr., F. F. Knapp, Jr., R. Shaw, S. Trowbridge, J. Chan, T. Spike, Y. C. Lu, R. Shapiro, D. Raulston, P. Chang, and H. Emery. 1974. Studies in sterol synthesis. *J. Am. Oil Chem. Soc.* **51,** A516.
11. Schroepfer, G. J., Jr. 1974. Metabolism of sterols and sphingolipids—major constituents of the membranes of eukaryotes. Abstracts, Southwest Regional A.C.S. Meeting (Houston, Texas) A23.
12. Trowbridge, S., Y. C. Lu, R. Shaw, J. Chan, and T. Spike. 1975. The enzymatic removal of carbon atom 32 of cholesterol precursors. *Federation Proc.* **34:** 560 (Abst.).
13. Schroepfer, G. J., Jr., R. A. Pascal, Jr., and A. A. Kandutsch. 1979. Inhibition of sterol synthesis in animal cells by 15-oxygenated sterols with the unnatural *cis*-C-D ring junction: 5α,14β-cholest-7-en-15β-ol-3-one and 5α,14β-cholest-7-en-15α-ol-3-one. *Biochem. Pharmacol.* **28:** 249–252.
14. Knapp, F. F., Jr., M. S. Wilson, and G. J. Schroepfer, Jr. 1976. Mass spectral fragmentation of 5α-hydroxysteroids. *Chem. Phys. Lipids.* **16:** 31–59.
15. Knapp, F. F., Jr., and G. J. Schroepfer, Jr. 1975. Chemical synthesis, spectral properties, and chromatography of 4α-methyl and 4β-methyl isomers of (24*R*)-24-ethyl-5α-cholestan-3β-ol and (24*S*)-24-ethyl-cholesta-5,22-dien-3β-ol. *Steroids.* **26:** 339–357.
16. Rieche, A., and H. Gross. 1960. α,α′-Dialkoxy- und -aryloxy-dimethyläther aus symm. Dichlordimethyläther. *Chem. Ber.* **93:** 259–264.
17. Van Es, A., and R. Stevens. 1964. Mixed carboxylic acid anhydrides. I. Acetic-formic anhydride. *Rec. Trav. Chim.* **83:** 863–872.
18. House, H. O., L.. J. Czuba, M. Gall, and H. D. Olmstead. 1969. The chemistry of carbanions. XVIII. Preparation of trimethylsilyl enol ethers. *J. Org. Chem.* **34:** 2324–2336.
19. Tomboulian, P., and K. Stehower. 1968. Chemistry of trityllithium. Condensation with benzophenone in tetrahydropyran. *J. Org. Chem.* **33:** 1509–1512.
20. Huntoon, S., B. Fourcans, B. N. Lutsky, E. J. Parish, H. Emery, F. F. Knapp, Jr., and G. J. Schroepfer, Jr. 1978. Chemical syntheses, spectral properties, and metabolism of 5α-cholest-8(14)-en-3β,15β-diol and 5α-cholest-8(14)-en-3β,15α-diol. *J. Biol. Chem.* **253:** 775–782.
21. Knight, J. C., P. D. Klein, and P. A. Szczepanik. 1966. The synthesis of tritium-labeled 14α-methyl-5α-cholest-7-en-3β-ol and its enzymatic demethylation. *J. Biol. Chem.* **241:** 1502–1508.
22. 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.
23. Spike, T. E., J. A. Martin, S. Huntoon, A. H-J. Wang, F. F. Knapp, Jr., and G. J. Schroepfer, Jr. 1978. Sterol synthesis. Chemical synthesis, structure determination and metabolism of 14α-methyl-5α-cholest-7-en-3β,15β-diol and 14α-methyl-5α-cholest-7-en-3β,15α-diol. *Chem. Phys. Lipids.* **21:** 31–58.
24. Woodward, R. B., A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly. 1957. The synthesis of lanosterol (lanostadienol). *J. Chem. Soc.* 1131–1144.

Downloaded from www.jlr.org by guest, on June 19, 2012

25. Knight, J. C., J. L. Belletire, and G. R. Pettit. 1967. Steroids and related natural products. Part XLII. 14α-Methyl cholestanes. *J. Chem. Soc.* 2427–2432.
26. Fried, J., J. W. Brown, and L. Borkenhagen. 1965. 32-Oxygenated lanostane derivatives from 3β-acetoxy-Δ^7-lanostene via 7α,8α-epoxides. *Tetrahedron Lett.* 2499–2504.
27. Fried, J., A. Dudowitz, and J. W. Brown. 1968. Enzymatic conversion of 32-oxygenated Δ^7-lanosterol derivatives and of $\Delta^{8(14)}$-4,4-dimethyl-cholestenol to cholesterol. *Biochem. Biophys. Res. Commun.* **32:** 568–574.
28. Akhtar, M., C. W. Freeman, D. C. Wilton, R. B. Boar, and D. B. Copsey. 1977. The pathway for the removal of the 15α-methyl group of lanosterol. The role of lanost-8-en-3β,32-diol in cholesterol biosynthesis. *Bioorg. Chem.* **6:** 473–481.
29. Spike, T. E., A. H-J. Wang, I. C. Paul, and G. J. Schroepfer, Jr. 1974. Structure of a potential intermediate in cholesterol biosynthesis. *J. Chem. Soc., Chem. Commun.* 477–478.
30. Martin, J. A., S. Huntoon, and G. J. Schroepfer, Jr. 1970. Enzymatic conversion of 14α-methyl-cholest-7-en-3β,15ξ-diol to cholesterol. *Biochem. Biophys. Res. Commun.* **39:** 1170–1174.
31. Bhacca, N. S., and D. H. Williams. 1964. Applications of NMR Spectroscopy in Organic Chemistry. Illustrations from the Steroid Field. Holden-Day, San Francisco, CA. 13–24.
32. Fieser, L. F., and M. Fieser. 1959. Steroids. Reinhold Publishing Company, New York, NY. 253.
33. Schroepfer, G. J., Jr., and I. D. Frantz, Jr. 1961. Conversion of Δ^7-cholesterol-4-C^{14} and 7-dehydrocholesterol-C^{14} to cholesterol. *J. Biol. Chem.* **236:** 3137–3140.
34. Cook, R. P. 1958. Cholesterol. Chemistry, Biochemistry, and Pathology. Academic Press, New York, NY. 92.
35. Kammereck, R., W-H. Lee, A. Paliokas, and G. J. Schroepfer, Jr. 1967. Thin-layer chromatography of sterols on neutral alumina impregnated with silver nitrate. *J. Lipid Res.* **8:** 282–284.
36. Anastasia, M., A. Fiecchi, G. Cighetti, and G. Galli. 1977. Synthesis of cholestanes containing an oxygenated 14α-methyl group. *J. Chem. Soc. Perkin I.* 700–702.

Downloaded from www.jlr.org by guest, on June 19, 2012