Sterol synthesis. A simplified method for the synthesis of 32-oxygenated derivatives of 24,25-dihydrolanosterol

Edward J. Parish and George J. Schroepfer, Jr.[1]

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

Abstract A simplified method is described for the preparation of 14α-hydroxymethyl-4,4-dimethyl-5α-cholest-8-en-3β-ol, 14α-hydroxymethyl-4,4-dimethyl-5α-cholest-7-en-3β-ol, and 14α-hydroxymethyl-4,4-dimethyl-5α-cholest-6-en-3β-ol from commercial lanosterol. This method represents a modification of the approach introduced by Fried and coworkers (Fried, J., J. W. Brown, and L. Borkenhagen. 1965. *Tetrahedron Lett.* 2499–2504).—**Parish, E. J., and G. J. Schroepfer, Jr.** Sterol synthesis. A simplified method for the synthesis of 32-oxygenated derivatives of 24,25-dihydrolanosterol. *J. Lipid Res.* 1981. **22:** 859–868.

Supplementary key words lanosterol · cholesterol

The biosynthesis of cholesterol from lanosterol (4α,4β,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol) requires the removal of the three "extra" methyl groups at carbon atoms 4 and 14. The initial reaction in the removal of each of these carbon atoms has been considered to involve an oxygen-dependent hydroxylation to yield the corresponding hydroxymethyl derivative (1–9). In addition to their probable intermediary role in the biosynthesis of cholesterol from 14α-methyl substituted sterol precursors of cholesterol, a number of 14α-hydroxymethyl sterols have recently been shown to inhibit sterol biosynthesis in animal cells in culture (10–12) and may play a role in the normal regulation of sterol biosynthesis. For further exploration of these matters, we required authentic samples of a number of 14α-hydroxymethyl derivatives of 24,25-dihydrolanosterol. This communication concerns a simplified procedure, based upon the approach introduced by Fried, Brown, and Borkenhagen (13) for the synthesis of lanost-7-ene-3β,32-diol, for the preparation of the 32-hydroxy derivatives of lanost-8-en-3β-ol, lanost-7-en-3β-ol, and lanost-6-en-3β-ol. In addition, spectroscopic characterization of these compounds and of the various intermediates in their synthesis is presented herein.

EXPERIMENTAL PROCEDURES AND RESULTS

General methods

Melting points (mp) were recorded in sealed, evacuated capillary tubes using a Thomas Hoover melting point apparatus. Optical rotations were measured using a JASCO DIP-4 digital polarimeter with $CHCl_3$ solutions of the sterols. Infrared (IR) spectra were recorded on a Beckman IR-9 spectrometer using KBr pellets. Gas–liquid chromatographic (GLC) analyses were performed using a Hewlett-Packard Model 402 unit equipped with flame ionization detectors. The columns (1.8 m × 6 mm) were packed with 3% OV-17 or 3% OV-1 on Gas-Chrom Q (100–120 mesh; Applied Science Laboratories, Inc., State College, PA). Low resolution mass spectral (MS) analyses were made using an LKB-9000S spectrometer under operating conditions described previously (14, 15) and the results are presented in terms of relative intensity (percentage of base peak) along with probable mode of origin. High resolution MS analyses were recorded on a Varian CH-5 spectrometer (courtesy of Professor C. C. Sweeley). Trimethylsilyl (TMS) ether derivatives were prepared as previously described (16). Analytical thin-layer chromatography (TLC) was performed on plates of silica gel G (Analtech, Newark, DE). Components on the plates were visualized after spraying with molybdic acid (17). Solvent systems for TLC were as follows: SS-1, 50% ethyl acetate in toluene; SS-2, 50% ethyl acetate in hexane; SS-3, 35% ethyl acetate in chloroform; SS-4, 50% ether in toluene;

Abbreviations: IR, infrared; GLC, gas-liquid chromatography; MS, mass spectral; TLC, thin-layer chromatography; MPLC, medium pressure liquid chromatography; NMR, nuclear magnetic resonance; TMS, trimethylsilyl.

[1] To whom inquiries should be directed.

SS-5, 50% ether in hexane; SS-6, chloroform; SS-7, toluene; SS-8, 5% ether in toluene; SS-9, 10% ether in hexane; SS-10, 5% ethyl acetate in toluene; SS-11, 10% ether in toluene; SS-12, 10% ethyl acetate in toluene; and SS-13, chloroform–ether–acetic acid 97:2.5:0.5. Medium pressure liquid chromatography (MPLC) was carried out on columns of silica gel (0.030–0.063 mm). Nuclear magnetic resonance (NMR) spectra were recorded on $CDCl_3$ solutions of the sterols using a Varian EM-390 spectrometer operating at 90 MHz and using tetramethylsilane as an internal standard. Peaks are reported as ppm (δ) downfield from the internal standard. Calculations of the C-18 and C-19 methyl resonances were made according to Zurcher (18).

Materials

Pyridine hydrochloride was prepared by passing anhydrous HCl through a solution of 25% pyridine in anhydrous ether (200 ml) for 10 min. The resulting precipitate was collected, washed with anhydrous ether, and dried in a vacuum dessicator over P_2O_5. Commercially available "lanosterol" (ICN Pharmaceuticals, Inc., Cleveland, OH), after three recrystallizations from acetone–water, was found to be a mixture of lanosterol (48%) and 24,25-dihydrolanosterol (52%) upon GLC analysis (3% OV-17; 250°C).

Lanost-8-en-3β-ol (I) from commercial lanosterol

Compound I (**Fig. 1**) was prepared from commercial lanosterol by catalytic hydrogenation, using a modification of the approach of Marker, Wittle, and Mixon (19) for the preparation of the acetate derivative of I from lanosteryl acetate. Platinum oxide (3.5 g) was added to a solution of recrystallized commercial lanosterol (20.0 g) in acetic acid (200 ml) at ~80°C and the resulting mixture was hydrogenated (55 psi) for 4 hr. After removal of the catalyst by filtration,[2] the solvent was evaporated to dryness under reduced pressure and the resulting residue was recrystallized twice from acetone–water to give I (18.2 g; 91% yield); mp, 146.0–147.5°C (lit., 148°C (19) and 146°C (20)). GLC analysis (3% OV-17; 250°C) indicated a purity of 98.5%.

Mixture of lanost-8-en-3β-ol (I) and lanost-7-en-3β-ol (II)

A mixture of I and II was prepared by treatment of I with dry HCl gas in $CHCl_3$ by the approach introduced by Marker et al. (19) for the case of the acetate derivative of I.

Compound I (25.0 g; 58.4 mmol) in $CHCl_3$ (1,500 ml) was treated with dry HCl gas for 2 hr at 25°C. The resulting mixture was covered and allowed to stand for an additional 2 hr at 25°C. The residue obtained upon evaporation of the solvent under reduced pressure was recrystallized once from acetone–water (containing ~10% NH_4OH)[3] and twice from acetone–water to give a mixture (21.9 g; 88% yield) of I (60%) and II (40%) as indicated by GLC analysis (3% OV-17; 250°C). The ratio of I to II was found to be unaffected by temperature, i.e., isomerizations carried out at −30°C, 0°C, and 25°C (HCl gas passed through the reaction mixture for 15 min at the specified temperature with maintenance at the same temperatures for an additional 2 hr) all gave the same ratio of I to II. Gaylor (21) reported that treatment of the acetate derivative of I with HCl at 25°C gave a mixture of the acetate derivatives of I (70%) and II (30%), as determined by optical rotation studies.

Epoxidation of mixture of lanost-8-en-3β-ol (I) and lanost-7-en-3β-ol (II)

To the mixture of I and II (5.00 g; 11.7 mmol) from above in CH_2Cl_2 (350 ml) were successively added $NaHCO_3$ (2.0 g) and *m*-chloroperbenzoic acid (2.5 g). After stirring for 36 hr at 25°C, ether was added and the resulting mixture was washed with 1N NaOH and water and then dried over anhydrous $MgSO_4$. The residue obtained upon evaporation of the solvent under reduced pressure was recrystallized from acetone–water to give a white crystalline solid (4.84 g). TLC analyses in six solvent systems (SS-1, SS-2, SS3, SS-4, SS-5, and SS-6) showed only one major component (~95%). GLC analysis (3% OV-17; 270°C) of the TMS derivative showed two major components (60% and 40% corresponding to the 8α,9α-epoxide (III) and 7α,8α-epoxide (IV), respectively, which comprised ~96% of the product. NMR analysis showed multiplets at 3.22 (C-3α-H) and 3.45 (0.4 H; C-7β-H).[4]

Lithium-ethylenediamine reduction of the mixture of epoxides III and IV

The mixture of epoxides III and IV (5.50 g; 12.4 mmol), obtained as described above, was dissolved in

[2] The catalyst was recovered for reutilization by successive washing with ethyl acetate, ethanol, and water. The resulting residue was heated at 120°C for 5 hr.

[3] The NH_4OH was included to neutralize any residual HCl. Failure to completely remove all traces of HCl will result in decreased yields of the desired epoxides in the following step.

[4] The resonance of the C-7 proton in 7α,8α-epoxy-lanostan-3β-ol has been reported at 3.47 ppm (13), at 3.3 ppm (22), and at 3.35 ppm (23) in the spectra of 3β-acetoxy-7α,8α-epoxy-14α-methyl-5α-cholestane.

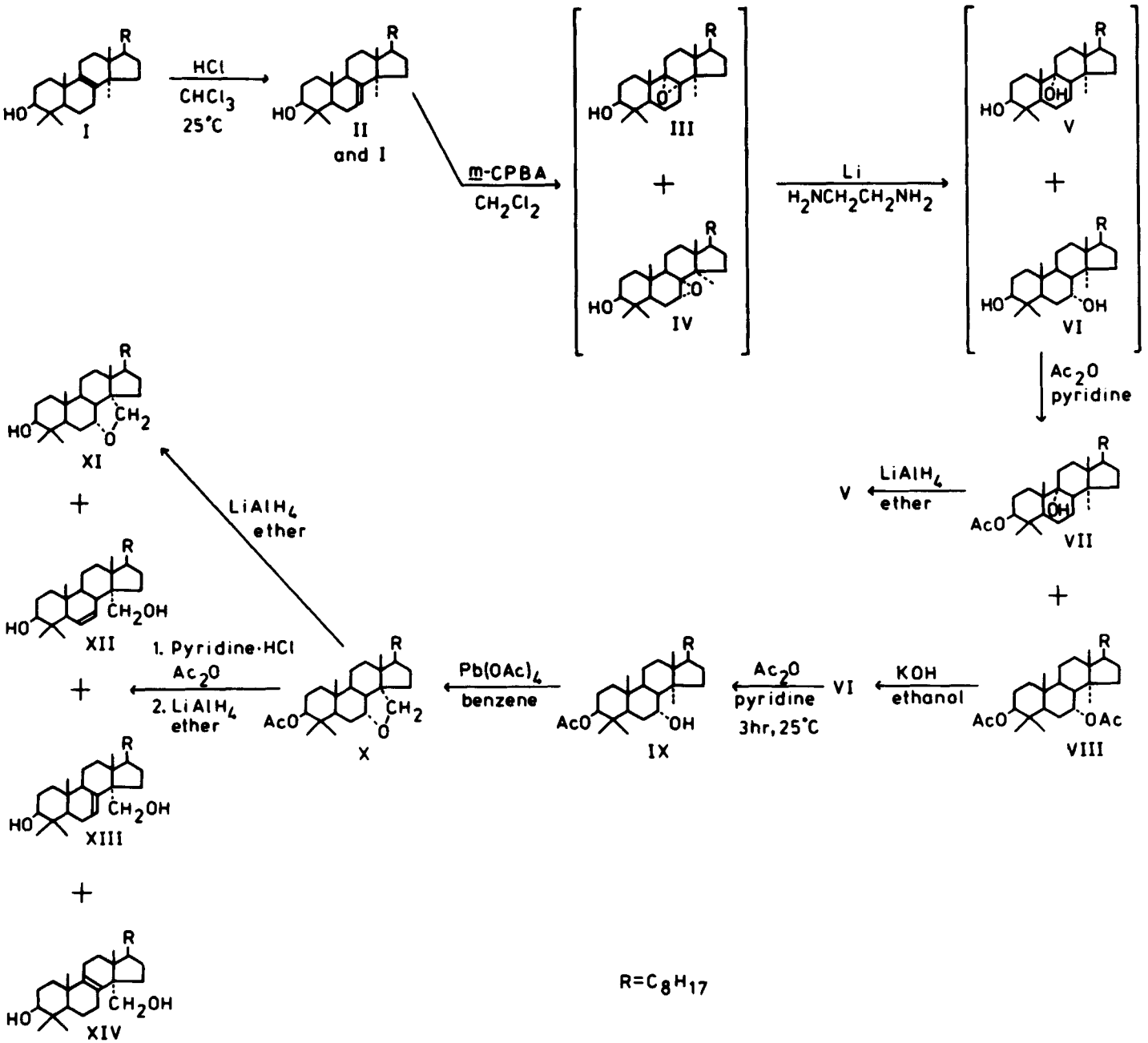

Fig. 1. Chemical synthesis of lanostene-6-ene-3β,32-diol (XII), lanost-7-ene-3β,32-diol (XIII), and lanost-8-ene-3β,32-diol (XIV).

freshly distilled ethylenediamine (125 ml) with gentle warming (~50°C). Lithium (1.5 g) was added in small portions with stirring at 25°C until a blue color persisted. The mixture was vigorously stirred at 25°C for an additional 15 min. Methanol was then added dropwise until the reagent was decomposed. Additional methanol was added until complete solution resulted. Water was added to precipitate the product which was collected, washed with water, and dried in a vacuum dessicator over P_2O_5 to give the crude product (5.05 g). Analysis by TLC (SS-1) showed two major components with R_f values of 0.51 and 0.71. The less polar component had the same mobility as that of I and II. The more polar component had the same chromatographic mobility as that of lanostane-3β,9α-diol (V) and lanostane-3β,7α-diol (VI) (vide infra). GLC analysis (3% OV-17; 260°C) of the crude product indicated the presence of four components with retention times corresponding to those of I (16.2%), II (9.1%), V (50.5%), and VI (24.2%).

The crude product was subjected to silica gel (60–200 mesh) column (60 cm × 2 cm) chromatography using 10% ethyl acetate in toluene as the eluting solvent (fraction size, 20 ml). The contents of fractions 23 through 28 were pooled and, after evaporation of solvent under reduced pressure, gave a mixture (1.20 g) composed of I (64%) and II (36%) as indicated by the results of GLC analyses. The contents of fractions 31 through 41 were pooled and, after evaporation of the solvent under reduced pressure, gave a mixture (3.75 g) composed of V (68%) and VI (32%) as indicated by the results of GLC analyses.

Attempted reductions of the mixture of epoxides with lithium and ethylamine according to Fried et al. (13) gave mixed results in small-scale (<1.0 g) reactions and very poor results on two large-scale (>4 g) runs. The report by Brown, Ikegami, and Kawakami (24) of the superiority of the use of ethylenediamine and lithium for this type of reaction prompted our utilization of this reagent. The procedure described above has been repeated three additional times with comparable yields.

3β-Acetoxy-lanostan-9α-ol (VII) and 3β,7α-bis-acetoxy-lanostane (VIII)

To the mixture of diols V and VI (5.00 g; 11.2 mmol), obtained as described above, in pyridine (75 ml) was added acetic anhydride (50 ml). After standing at 25°C overnight, the reaction mixture was poured into ice-water and extracted thoroughly with ether. The combined extracts were washed successively with water, cold aqueous 5% HCl, aqueous 5% $NaHCO_3$, and water, filtered, and evaporated to dryness under reduced pressure. The residue (4.81 g) was subjected to MPLC (60 psi; 100 cm × 2.5 cm) using 1.25% ether in toluene as the eluting solvent (flow rate, 5 ml per min; fraction size, 20 ml). The contents of fractions 134 through 189 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from acetone–water to give VIII (1.63 g; 28% yield); mp, 171–172°C (lit., 171.0–171.5°C (13) and 167–168°C (25)); IR, 1745 and 1034 cm^{-1}; NMR, 0.77 (s, 3H, C-18-CH_3; calc., 0.77), 0.88 (s, 3H, C-19-CH_3; calc. 0.91), 2.01 and 2.03 (s, 3H each, methyls of acetoxy functions), 4.53 (m, 1H, C-3-H), and 5.13 (m, 1H, C-7-H); MS: 530 (3%; M), 470 (34%; M – CH_3COOH), 455 (81%; M – CH_3 – CH_3COOH), 410 (21%; M – CH_3COOH – CH_3COOH), 395 (100%; M – CH_3 – CH_3COOH – CH_3COOH), 357 (4%; M – CH_3COOH – side chain), 330 (34%), 297 (9%; M – CH_3 – CH_3COOH – CH_3COOH), 270 (33%), and 255 (32%); $[\alpha]_D$ – 16.3° (*c*, 0.51), (lit., −16° (13) and −20° (25)). The product showed a single component on TLC in three solvent systems (SS-7, SS-8, and SS-9) and on GLC (3% OV-1 and 3% OV-17; 260°C).

The contents of fractions 207 through 268 were combined and, after evaporation of the solvent under reduced pressure, recrystallized from acetone–water to give VII (3.11 g; 57% yield); mp, 166–168°C (lit., 163–164°C (26) and 168–169°C (27)); IR, 3550, 1725, 1267, and 1035 cm^{-1}; NMR, 0.79 (s, 3H, C-18-CH_3; calc., 0.77), 1.04 (s, 3H, C-19-CH_3; calc., 1.05), 1.99 (s, 3H, methyl of acetoxy function), and 4.46 (m, 1H, C-3-H); MS: 488 (2%; M), 470 (17%; M – H_2O), 455 (58%; M – CH_3 – H_2O), 428 (14%; M – CH_3COOH), 413 (100%; M – CH_3 – H_2O-42), 410 (6%; M – H_2O – CH_3COOH), 395 (54%; M – CH_3 – H_2O – CH_3COOH), 357 (3%; M – H_2O – side chain), 315 (4%; M – CH_3COOH – side chain), 297 (4%; M – H_2O – CH_3COOH – side chain), 291 (30%), 289 (13%), and 203 (40%); $[\alpha]_D$ + 6.8° (*c*, 0.36) (lit., +7° (26) and +18° (27)). The product showed a single component on TLC in three solvent systems (SS-8, SS-9, and SS-10).

Lanostane-3β,7α-diol (VI) from 3β,7α-*bis*-acetoxy-lanostane (VIII)

To VIII (1.50 g; 2.80 mmol) in ethanol (65 ml) was added 21.7 N KOH (7.5 ml). The resulting mixture was heated under reflux for 6 hr and, after cooling to room temperature, poured into cold water. The precipitated sterol was collected, dried, and subjected to MPLC (100 psi; 118 cm × 1.5 cm) using 20% ethyl acetate in toluene as the eluting solvent (flow rate, 4 ml per min; fraction size, 20 ml). The contents of fractions 54 through 76 were pooled and, after evapora-

tion of the solvent under reduced pressure, recrystallized from acetone–water to give VI (1.15 g; 91% yield); mp, 166–167°C (lit., 163–165°C (25) and 163–166°C (13)); IR, 3450 and 1037 cm^{-1}; NMR, 0.79 (s, 3H, C-18-CH_3; calc. 0.78), 0.88 (s, 3H, C-19-CH_3; calc., 0.88), 3.24 (m, 1H, C-3-H), and 4.06 (m, 1H, C-7-H); MS: 446 (10%; M), 431 (19%; M – CH_3), 428 (15%; M – H_2O), 413 (18%; M – CH_3 – H_2O), 410 (4%; M – H_2O – H_2O), 395 (10%; M – CH_3 – H_2O – H_2O), 315 (8%; M – H_2O – side chain), 297 (7%; M – H_2O – H_2O – side chain), 288 (70%), 273 (32%), 270 (14%); 248 (32%), 235 (37%), and 222 (100%); $[\alpha]_D$ + 3.7° (*c*, 0.42) (lit., +5° (25) and +3.2° (13)). The product showed a single component on TLC in three solvent systems (SS-1, SS-3, and SS-4). GLC analyses (3% OV-1 and 3% OV-17; 270°C) of the free sterol and its *bis*-TMS derivative indicated a purity of 99.8%.

Lanostane-3β,9α-diol (V) from 3β-acetoxy-lanostan-9α-ol (VII)

To compound VII (250 mg; 0.51 mmol) in ether (25 ml) was added lithium aluminum hydride (0.5 g). After stirring for 3 hr at 25°C, the mixture was cooled to 0°C and ice was cautiously added to decompose the unreacted hydride. The mixture was poured into a saturated NH_4Cl solution and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined ether extracts were dried over anhydrous $MgSO_4$ and the residue (210 mg) obtained upon evaporation of the solvent under reduced pressure was recrystallized from acetone–water to give V (204 mg; 89% yield), mp, 135.5–136.5°C (lit., 135–136°C (26)); IR, 3480 and 1042 cm^{-1}; NMR, 0.79 (s, 3H, C-18-CH_3; calc. 0.77), 1.03 (s, 3H C-19-CH_3; calc., 1.03), and 3.19 (m, 1H, C-3-H); MS: 446 (2%; M), 431 (2%; M – CH_3), 428 (21%; M – H_2O), 413 (100%; M – CH_3 – H_2O), 410 (3%; M – H_2O – H_2O), 395 (37%; M – CH_3 – H_2O – H_2O), 315 (2%; M – H_2O – side chain), and 297 (3%; M – H_2O – H_2O – side chain); $[\alpha]_D$ + 2.8° (*c*, 0.38) (lit., + 2.5° (26)). The product showed a single component on TLC in three solvent systems (SS-1, SS-3, and SS-5).

3β-Acetoxy-lanostan-7α-ol (IX) from lanostane-3β,7α-diol (VI)

To compound VI (1.00 g; 2.24 mmol) in pyridine (20 ml) was added acetic anhydride (10 ml). After standing 3 hr at 25°C, the mixture was poured into ether (1,000 ml). The resulting mixture was washed successively with water, cold 5% aqueous HCl, 5% aqueous $NaHCO_3$, and water, and then dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was subjected to MPLC (60 psi; 100 cm × 2.5 cm) using 5% ether in toluene as the eluting solvent (fraction size, 20 ml). The contents of fractions 121 through 156 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from acetone–water to give IX (0.91 g; 84% yield); mp, 209–211°C (lit., 205–206°C (25, 28), 209.0–210.5°C (13), and 212°C (7)); IR, 3440, 1744, and 1045 cm^{-1}; NMR, 0.76 (s, 3H, C-18-CH_3; calc., 0.76), 0.89 (s, 3H, C-19-CH_3; calc., 0.90), 1.98 (s, 3H, methyl of acetoxy function), 4.06 (m, 1H, C-7-H), and 4.52 (m, 1H, C-3-H); MS: 488 (1%; M), 470 (22%; M – H_2O), 455 (82%; M – CH_3 – H_2O), 413 (3%; M – CH_3 – CH_3COOH), 410 (7%; M – H_2O – CH_3COOH), 395 (100%; M – CH_3 – H_2O – CH_3COOH), 380 (2%), 357 (2%; M – H_2O – side chain), 315 (6%; M – CH_3COOH – side chain), and 297 (5%; M – H_2O – CH_3COOH – side chain); $[\alpha]_D$ + 13.9° (*c*, 0.42) (lit., +14° (13, 25), +14.6° (7), and +13.8° (28)). The product showed a single component on TLC in three solvent systems (SS-9, SS-11, and SS-12). GLC analyses (3% OV-17 and 3% OV-1; 270°C) of the free sterol and its TMS derivative indicated a purity of 98%.

3β-Acetoxy-7α,32-epoxy-lanostane (X) from 3β-acetoxy-lanostan-7α-ol (IX)

Compound IX (1.00 g; 2.05 mmol) was dissolved in dry benzene (500 ml) and ~75 ml of the solvent was distilled off to remove any traces of water. Lead tetraacetate (5.0 g) was added and the resulting mixture was heated under reflux for 24 hr. After cooling to room temperature, a 20% aqueous KI solution (100 ml) was added. After the addition of a saturated solution of sodium thiosulfate (until the yellow precipitate had dissolved), the mixture was thoroughly extracted with ether. The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The resulting residue was subjected to MPLC (100 psi; 118 cm × 1.5 cm) using 5% ether in toluene as the eluting solvent (fraction size, 20 ml). The contents of fractions 30 through 43 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from acetone–water to give X (0.74 g; 74% yield); mp, 201–203°C (lit., 195–197°C (7), 181–183°C (28), and 202–204°C (13); IR, 1740, 1038, and 994 cm^{-1}; NMR, 0.80 (s, 3H, C-18-CH_3), 0.86 (s, 3H, C-19-CH_3), 1.98 (s, 3H, methyl of acetoxy function), 3.34 (d, 1H, C-32-H; J = 8 Hz), 3.98 (d, 1H, C-32-H; J = 8 Hz), 4.18 (m, 1H, C-7-H), and 4.49 (m, 1H, C-3-H); MS: 486 (3%; M), 471 (3%; M – CH_3), 456 (74%; M – CH_2O), 455 (100%; M – CH_2OH), 441 (11%; M – CH_3 – CH_2O), 413 (2% M – CH_2OH – 42), 395 (55%; M – CH_3COOH

– CH_2OH), 381 (14%; M – CH_3 – CH_2O – CH_3COOH), 373 (8%; M – side chain), 331 (42%; M – side chain – 42), 313 (4%; M – side chain – CH_3COOH), and 283 (12%; M – CH_2O – side chain – CH_3COOH); $[\alpha]_D$ + 25.3° (*c*, 0.47) (lit., +25° (7, 13) and +24° (28)). The product showed a single component on TLC in three solvent systems (SS-9, SS-11, and SS-12) and a purity of 99% on GLC analyses (3% OV-1 and 3% OV-17; 270°C).

7α,32-Epoxy-lanostan-3β-ol (XI) from 3β-acetoxy-7α,32-epoxy-lanostane (X)

To compound X (60 mg; 0.12 mmol) in ether (20 ml) was added lithium aluminum hydride (200 mg). After stirring for 3 hr at 25°C, the reaction mixture was cooled to 0°C and ice was cautiously added to decompose the unreacted hydride. The mixture was poured into a saturated NH_4Cl solution and thoroughly extracted with ether containing CH_2Cl_2 (15%). The combined extracts were dried over anhydrous $MgSO_4$ and the residue obtained upon evaporation of the solvent under reduced pressure was recrystallized from acetone–water to give XI (49 mg; 91% yield); mp, 179.5–181.5°C; IR, 3480, 1062, and 978 cm^{-1}; NMR, 0.77 (s, 3H, C-18-CH_3), 0.87 (s, 3H, C-19-CH_3), 3.22 (m, 1H, C-3-H), 3.32 (d, 1H, C-32-H; J = 8 Hz), 3.97 (d, 1H, C-32-H; J = 8 Hz), and 4.17 (m, 1H, C-7-H); MS: 444 (3%; M), 429 (3% M – CH_3), 426 (1%; M – H_2O), 414 (60%; M – CH_2O), 413 (100%; M – CH_2OH), 399 (12%; M – CH_2O – CH_3), 395 (33%; M – H_2O – CH_2OH), 331 (6%; M – side chain), 313 (3%; M – H_2O – side chain), 299 (58%); high resolution MS: 444.3992 (calc. for $C_{30}H_{52}O_2$: 444.3967); MS on TMS derivative, 516 (7%; M), 501 (6%; M – CH_3), 486 (35%; M – CH_2O), 485 (49%; M – CH_2OH), 471 (3%; M – CH_3 – CH_2O), 426 (5%; M – trimethylsilanol), 411 (2%; M – CH_3 – trimethylsilanol), 403 (3%; M – side chain), 396 (23%; M – CH_2O – trimethylsilanol), 395 (49%, M – CH_2OH – trimethylsilanol), 386 (60%), 381 (8%; M – CH_3 – CH_2O – trimethylsilanol), 298 (3%; M – CH_3 – trimethylsilanol – side chain), and 283 (6%; M – CH_2O – trimethylsilanol – side chain). The product showed a single component on TLC in three solvent systems (SS-1, SS-3, and SS-5) and a purity of 99% on GLC (3% OV-17 and 3% OV-1; 270°C) of the free sterol and its TMS derivative.

7α,32-Epoxy-lanostan-3β-ol (XI), lanost-6-ene-3β,32-diol (XII), lanost-7-ene-3β,32-diol (XIII), and lanost-8-ene-3β,32-diol (XIV) from 3β-acetoxy-7α,32-epoxy-lanostane (X)

Compound X (500 mg; 1.03 mmol) was heated under reflux for 15 hr with pyridine hydrochloride (1.0 g) in acetic anhydride (100 ml). After cooling to 25°C, the mixture was poured into ice-water and allowed to stand for 1.5 hr. The product was recovered by thorough extraction with ether and the combined extracts were washed successively with cold aqueous 5% HCl, 5% aqueous $NaHCO_3$, and water. The extract was dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The resulting residue, after further drying over P_2O_5 in vacuo, was dissolved in ether (60 ml), and lithium aluminum hydride (60 mg) was added. After standing for 2 hr at 25°C, the mixture was cooled to 0°C and ice was cautiously added to decompose the unreacted hydride. The resulting mixture was poured into a saturated NH_4Cl solution and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. Analysis by TLC (SS-1) indicated two major components (R_f 0.57 and 0.49). The crude product was subjected to MPLC (100 psi; 118 cm × 1.5 cm) using 25% ethyl acetate in toluene as the eluting solvent (fraction size, 20 ml). The contents of fractions 17 through 22 (corresponding to the material of R_f 0.57 on TLC) were pooled and evaporated to dryness under reduced pressure. The residue (210 mg) was subjected to preparative TLC (SS-3) on silica gel PF impregnated with $AgNO_3$ (10%). The major component (least polar, R_f 0.56) was extracted with ether and recrystallized from acetone–water to give XI (62.3 mg; 13.6% yield); mp, 179.5–181.5°C; $[\alpha]_D$ + 10.0° (*c*, 0.22). The IR and NMR spectra were identical with those of XI which was prepared, as described above, by direct hydride reduction of X. The MS and GLC properties of the free sterol and its TMS derivative were also identical to those of XI (prepared as described above). The product showed a single component on TLC in three solvent systems (SS-1, SS-3, and SS-5).

The more polar (R_f 0.31) component from the preparative TLC was extracted with ether and recrystallized from methanol–water to give XII (30.6 mg; 6.2% yield); mp, 191.5–192.5°C (lit., 190–192°C (29)); IR, 3400, 1068, and 1031 cm^{-1}; NMR, 0.66 (s, 3H, C-18-CH_3; calc., 0.65), 0.87 (s, 3H, C-19-CH_3; calc., 0.87), 3.35 (m, 2H, C-3-H and C-32-H), 4.17 (d, 1H, C-32-H; J = 12 Hz), 5.66 and 5.84 (d, 1H each, C-7-H and C-6-H; J = 12 Hz); MS: 444 (3%; M), 426 (29%; M – H_2O), 414 (47%; M – CH_2O), 413 (100%; M – CH_2OH), 408 (6%; M – H_2O – H_2O), 399 (10%; M – CH_3 – CH_2O), 395 (86%; M – CH_2OH – H_2O), 393 (27%; M – CH_3 – H_2O – H_2O), 381 (6%; M – CH_3 – H_2O – CH_2O), 331 (4%; M – side chain), 313 (10%; M – H_2O – side chain), 301 (9%; M – CH_2O – side chain), 300 (12%; M – side chain – CH_2OH), 299 (32%), 283 (89%;

M – H_2O – CH_2O – side chain), and 259 (17%); MS on *bis*-TMS derivative: 588 (3%; M), 573 (8%; M – CH_3), 498 (54%; M – trimethylsilanol), 485 (19%; M – $CH_2OSi(CH_3)_3$), 408 (70%; M – trimethylsilanol – trimethylsilanol), 395 (100%; M – trimethylsilanol – $CH_2OSi(CH_3)_3$), 393 (56%; M – CH_3 – trimethylsilanol – trimethylsilanol), 385 (7%; M – trimethylsilanol – side chain), 367 (10%), 295 (20%; M – trimethylsilanol – trimethylsilanol – side chain); $[\alpha]_D$ – 28.6° (*c*, 0.22) (lit., −29° (29)). The product showed a single component on TLC in three solvent systems (SS-1, SS-3, and SS-4) and a purity of 98.5% on GLC (3% OV-17 and 3% OV-1; 270°C) analyses of the free sterol and its TMS derivative.

The contents of fractions 24 through 32 from the MPLC run were pooled and evaporated to dryness under reduced pressure. The resulting residue (296 mg) showed two components (R_f 0.11 and 0.08) on TLC (SS-13) and on GLC (3% OV-17; 260°C). The mixture was subjected to silica gel (60–200 mesh) column (130 cm × 2 cm) chromatography using SS-13 as the eluting solvent at a flow rate of 4 ml per min (fraction size, 20 ml). The contents of fractions 41 through 53 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from methanol–water to give XIII (174 mg; 38% yield); mp, 207.0–208.5°C (lit., 207–209°C (29), 201–203°C (28), 208–210°C (7), 201°C (8), 204–205°C (30), and 202–204°C (4)); IR, 3450, 1059, and 1031 cm^{-1}; NMR, 0.73 (s, 3H, C-18-CH_3; calc., 0.72), 0.91 (s, 3H, C-19-CH_3; calc., 0.91), 3.22 (m, 2H, C-3-H and C-32-H), 3.65 (d, 1H, C-32-H; J = 11 Hz), and 5.37 (m, 1H, C-7-H); MS: 444 (2%; M), 429 (1%; M – CH_3), 426 (4%; M – H_2O), 414 (100%; M – CH_2O), 413 (62%; M – CH_2OH), 399 (17%; M – CH_3 – CH_2O), 395 (53%; M – H_2O – CH_2OH), 381 (10%; M – CH_3 – H_2O – CH_2O), 313 (9%; M – H_2O – side chain), 301 (8%; M – CH_2O – side chain), 299 (9%), and 283 (9%; M – H_2O – CH_2O – side chain); MS on *bis*-TMS derivative: 588 (1%; M), 573 (3%; M – CH_3), 498 (7%; M – trimethylsilanol), 485 (53%; M – $CH_2OSi(CH_3)_3$), 483 (5%; M – CH_3 – trimethylsilanol), 408 (2%; M – trimethylsilanol – trimethylsilanol), 395 (100%; M – trimethylsilanol – $CH_2OSi(CH_3)_3$), 393 (8%; M – CH_3 – trimethylsilanol – trimethylsilanol), 385 (3%; M – trimethylsilanol – side chain), 295 (4%; M – trimethylsilanol – trimethylsilanol – side chain), 241 (7%), and 227 (7%); $[\alpha]_D$ + 13.8° (*c*, 0.31) (lit., + 13.5° (29), +10.7° (7), +10° (30), and +12° (28)). The product showed a single component on TLC in four solvent systems (SS-1, SS-3, SS-4, and SS-13) and a purity of 99% as judged by GLC (3% OV-17 and 3% OV-1; 270°C) analyses of the free sterol and its *bis*-TMS derivative.

The contents of fractions 56 through 64 of the silica gel gravity column chromatography were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from methanol–water to give XIV (102 mg; 22% yield); mp, 161–163°C (lit., 159–161°C (29) and 174–175°C (7)); IR, 3400, 1040, and 1020 cm^{-1}; NMR, 0.72 (s, 3H, C-18-CH_3; calc., 0.76), 1.03 (s, C-19-CH_3; calc., 1.03), 3.21 (d, 1H, C-32-H; J = 11 Hz), 3.28 (m, 1H, C-3-H), and 3.66 (m, 1H, C-32-H; J = 11 Hz); MS: 444 (1%; M), 426 (28%; M – H_2O), 414 (100%; M – CH_2O), 413 (60%; M – CH_2OH), 399 (8%; M – CH_3 – CH_2O), 395 (49%; M – H_2O – CH_2OH), 392 (16%), 381 (11%; M – CH_3 – H_2O – CH_2O), 313 (12%; M – H_2O – side chain), 301 (6%; M – CH_2O – side chain), 299 (11%), 283 (5%; M – H_2O – CH_2O – side chain); MS on *bis*-TMS derivative: 588 (0.5%; M), 573 (2%; M – CH_3), 498 (2%; M – trimethylsilanol), 485 (54%; M – $CH_2OSi(CH_3)_3$), 483 (6%; M – CH_3 – trimethylsilanol), 408 (2%; M – trimethylsilanol – trimethylsilanol), 395 (100%; M – trimethylsilanol – $CH_2OSi(Ch_3)_3$), 393 (6%; M – CH_3 – trimethylsilanol – trimethylsilanol), 385 (19%; M – trimethylsilanol – side chain), 295 (2%; M – trimethylsilanol – trimethylsilanol – side chain), 241 (6%), and 227 (8%); $[\alpha]_D$ + 43.7° (*c*, 0.33) (lit., +43° (29) and +52.1° (7)). The product showed a single component on TLC in four solvent systems (SS-1, SS-3, SS-4, and SS-13) and a purity in excess of 97% on GLC (3% OV-17 and 3% OV-1; 270°C) analyses of the free sterol and its *bis*-TMS derivative.

DISCUSSION

Several routes have been described for the chemical synthesis of lanost-7-ene3β,32-diol. One approach is that described by Barton and coworkers (28, 31–33). The ultimate starting material for this route is 3β-acetoxy-lanost-8-ene, which can be prepared from crude lanosterol obtained from lanolin (19). Upon oxidation of the Δ^8-steryl acetate, a complex mixture is obtained from which 3β-acetoxy-lanost-8-en-7-one can be isolated (either directly or via oxidation of the proposed $\Delta^{7,9(11)}$-steryl intermediate) (19, 34–38). Reduction of the Δ^8-7-keto-steryl acetate with lithium in liquid ammonia, followed by chromatography, gave, in unspecified yield, 3β-acetoxylanostan-7-one (25). Catalytic reduction of the latter compound afforded 3β-acetoxy-lanostan-7α-ol in 69% yield (28). 3β-Acetoxy-lanostan-7α-yl nitrite was prepared, in 92% yield, from the 7α-hydroxysteryl ester by treatment with nitrosyl chloride (28). Photolysis of the nitrite ester gave 3β-acetoxy-32-hydroxyimino-lano-

stan-7α-ol in 60% yield (28). Treatment of the oxime with methanesulfonyl chloride in pyridine afforded 3β-acetoxy-32-nitrilo-lanostan-7α-yl methanesulfonate in 89% yield (28). The latter compound, upon heating with dry collidine, gave 3β-acetoxy-lanost-7-en-32-onitrile in 79% yield (28). Reduction of the nitrile with lithium aluminum hydride gave 3β-hydroxy-lanost-7-en-32-al in 63% yield (28). Reduction of the 3β-acetate derivative, prepared from the free sterol, with lithium aluminum hydride afforded lanost-7-ene-3β,32-diol in 88% yield (28). Thus, by this approach, lanost-7-ene-3β,32-diol can be obtained, in 11 steps, from 3β-acetoxy-lanost-8-ene. The yield of the Δ^7-3β,32-diol from 3β-acetoxy-lanostan-7-one by this route is ~15%. The overall yield from the ultimate starting material (3β-acetoxy-lanost-8-ene) cannot be calculated due to lack of specification of the yields of the reactions involved in the conversion of the latter compound to 3β-acetoxy-lanostan-7-one. Attempts to prepare lanost-8-ene-3β,32-diol by modifications of the approach outlined above were unsuccessful (28). This scheme was subsequently improved by the finding that photolysis of 3β-acetoxy-lanostan-7α-yl nitrite in the presence of oxygen gave 3β-acetoxy-7α-lanostan-32-yl nitrate in 44% yield (32). Treatment of the nitrate ester with methanesulfonyl chloride in pyridine afforded a product (not isolated) which, upon treatment with basic alumina, gave 3β-acetoxy-lanostan-7-en-32-yl nitrate in 86% yield (32). Reduction of the nitrate ester with zinc dust in acetic acid gave 3β-acetoxy-lanost-7-en-32-al in 84% yield (32).

Concomitant with the development of the Barton nitrite ester photolytic approach outlined above, Fried et al. (13) described an alternative approach for the synthesis of lanost-7-ene-3β,32-diol. The starting material for this synthesis was 3β-acetoxy-lanost-7-ene. This compound can be prepared from its corresponding Δ^8-isomer (prepared from crude lanosterol obtained from lanolin (19)) by treatment with dry HCl in chloroform (19). Under these conditions, a mixture of the Δ^8 and Δ^7 isomers is obtained from which the Δ^7-steryl acetate can be isolated, in unspecified yield, by chromic acid oxidation of the mixture under controlled conditions followed by repeated crystallization of the crude product (19). The Fried synthesis is based upon conversion of the Δ^7-steryl acetate to the corresponding 7α,8α-epoxide, reduction of the latter compound with lithium in ethylamine to give lanostane-3β,7α-diol, selective acetylation of the 3β,7α-dihydroxysterol to give the 3β-monoacetate, treatment of the latter compound with lead-tetraacetate to afford the cyclic 7α,32-oxide, and acetolytic cleavage of the 7α,32-epoxide to give 3β,32-diacetoxy-lanost-7-ene (13). This important synthetic work was, unfortunately, presented only in preliminary form. The results of subsequent utilization of this method in the lanostane series indicated that the acetolytic cleavage of the 7α,32-oxide yields not only the 32-oxygenated-Δ^7-sterol derivative, but also products to which the Δ^8 (7, 8) and Δ^6 (8) isomeric structures were assigned. However, in these cases, presentation of experimental details and characterization of the products was limited. The approach of Fried et al. (13) has also been applied to the case of the synthesis of 14α-hydroxymethyl-5α-cholest-7-en-3β-ol and its derivatives (10, 22, 23). Knight, Belletire, and Pettit (22) reported that treatment of 3β-acetoxy-7α,32-epoxy-14α-methyl-5α-cholestane with acetic anhydride and pyridine hydrochloride gave a complex mixture from which 3β-acetoxy-14α-acetoxymethyl-5α-cholest-7-ene was isolated in low yield. The results of subsequent studies in this laboratory (10, 23) demonstrated that 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 afforded 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.

The approach introduced by Fried et al. (13) for the synthesis of 32-oxygenated derivatives of lanostane forms the basis for the modified and expanded synthetic work described herein. Lanost-8-en-3β-ol (I) was prepared from commercial lanosterol by the method of Marker et al. (19). Treatment of the Δ^8-sterol with dry HCL in chloroform, under the conditions described by Marker et al. (19), gave a 60:40 mixture of lanost-8-en-3β-ol (I) and lanost-7-en-3β-ol (II). Rather than attempting to separate these isomers (or to isolate the Δ^7-sterol after selective oxidation of the Δ^8-sterol (19)), the mixture of the Δ^8- and Δ^7-sterols was epoxidized to give, in high yield, a mixture of the 8α,9α-epoxide (III) and the 7α,8α-epoxide (IV). Reduction of the mixture with lithium in ethylenediamine produced a mixture of lanost-8-en-3β-ol (I), lanost-7-en-3β-ol (II), lanostane-3β,9α-diol (V), and lanostane-3β,7α-diol (VI). The former two products were separated from the latter two products by silica gel column chromatography. Brown et al. (24) reported significant advantages of the replacement of ethylenediamine for ethylamine in lithium metal reduction of hindered and labile epoxides. In the present study, the use of ethylenediamine, rather than ethylamine, gave improved yields of the desired products of reduction, especially in large-scale syntheses. The mixture of the 3β,9α-diol and the 3β,7α-diol was treated with acetic anhydride

and pyridine at room temperature to give, after MPLC, 3β-acetoxy-lanostan-9α-ol (VII) and 3β,7α-*bis*-acetoxy-lanostane (VIII). The melting point of the 3β-acetoxy-9α-hydroxysterol (VII) was in reasonable agreement with values published by Fried, Brown, and Applebaum (26) and by Guest and Marples (27). The observed optical rotation (+6.8°) was in good agreement with that (+7°) reported by the former workers (26) but significantly different from that (+18°) reported by the latter workers (possibly due to the presence of a small amount of the highly dextrorotatory (27) 3β-acetoxy-lanost-9(11)-ene). In addition, reduction of VII with lithium aluminum hydride gave, in high yield, lanostane-3β,9α-diol (V) whose melting point and rotation were in close agreement with those reported by Fried et al. (26). Moreover, the results of IR, NMR, and MS analyses of V and VII, previously unreported, were in full agreement with the assigned structures. The melting points and optical rotations of the 3β,7α-diacetate (VIII) and of 3β,7α-dihydroxysterol (VI) (obtained in high yield by reduction of the diacetate with lithium aluminum hydride) were in reasonable agreement with values reported by Fried et al. (13) and Barton and Thomas (25). Moreover, the results of IR, NMR, MS analyses of VI and VIII, previously unreported (with the exception of the presence of the signal due to the C-7 olefinic proton in VI and VIII (13)), were compatible with the assigned structures.

Selective acetylation of the 3β,7α-dihydroxysterol (VI) afforded 3β-acetoxy-lanostan-7α-ol (IX) in 84% yield. Compound IX, characterized by standard spectral assays and by its melting point and optical rotation, was treated with lead tetraacetate in benzene to give, after MPLC and recrystallization from acetone–water, 3β-acetoxy-7α,32-epoxy-lanostane (X) in 74% yield. Compound X was characterized by its melting point, optical rotation, and the results of IR, NMR, and mass spectral analyses as well as by conversion, in 91% yield, to the previously undescribed 7α,32-epoxy-lanostan-3β-ol (XI). Compound XI was characterized by its melting point and by the results of IR, NMR, and high and low resolution mass spectral analyses as well as by the results of mass spectral analyses of the trimethylsilyl derivative of XI.

The 7α,32-epoxy-steryl acetate (X), upon treatment with pyridine hydrochloride in acetic anhydride followed by reduction of the crude product with lithium aluminum hydride, gave a mixture that was subjected to MPLC on a silica gel column. The nonpolar fraction from the MPLC was further subjected to preparative TLC on silica gel PF-$AgNO_3$. The least polar component was recovered and recrystallized to afford XI and the more polar component was recovered and recrystallized to give lanost-6-ene-3β,32-diol (XII). The more polar fraction from the MPLC was subjected to further chromatography on silica gel column chromatography. A less polar fraction afforded, after recrystallization, lanost-7-ene-3β,32-diol (XIII) while a more polar fraction gave, after recrystallization, lanost-8-ene-3β,32-diol (XIV). The yields of pure XI, XII, XIII, and XIV were 13.6, 6.2, 38 and 22%, respectively. The Δ^6 (XII), Δ^7 (XIII), and Δ^8 (XIV) sterols were characterized by determination of melting point and optical rotation and by the results of IR, NMR, and mass spectral analyses, as well as by the results of mass spectral analyses of the corresponding trimethylsilyl derivatives.

The synthetic scheme presented in this paper provides a simplified method for the preparation of lanost-7-ene-3β,32-diol from commercially available lanosterol. Moreover, this approach also yields the corresponding Δ^6- and Δ^8-isomers. Lanost-8-ene-3β,32-diol and lanost-7-ene-3β,32-diol are of importance not only for use in studies of the biosynthesis of cholesterol but also, along with lanost-6-ene-3β,32-diol, in studies of the inhibition of sterol biosynthesis by 14α-hydroxymethyl sterols. The overall yields of pure lanost-6-ene-3β,32-diol, lanost-7-ene-3β,32-diol, and lanost-8-ene-3β,32-diol from lanost-8-en-3β-ol, by this procedure, were approximately 0.6%, 3.4% and 2.0%, respectively.

This research was supported in part by a grant from the National Institutes of Health (HL-22532).

Manuscript received 12 January 1981 and in revised form 6 April 1981.

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. *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. 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.
5. 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. R. Soc. London Ser. B.* **180:** 125–146.
6. 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.
7. 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.

8. 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.
9. Pascal, R. A., Jr., P. Chang, and G. J. Schroepfer, Jr. 1980. Concerning possible mechanisms of demethylation of 14α-methyl sterols in cholesterol biosynthesis. *J. Am. Chem. Soc.* **45:** 4034–4037.
10. 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.
11. Gibbons, G. F., C. R. Pullinger, H. W. Chen, W. K. Cavenee, and A. A. Kandutsch. 1980. Regulation of cholesterol biosynthesis in cultured cells by probable natural precursor sterols. *J. Biol. Chem.* **255:** 394–400.
12. Schroepfer, G. J., Jr., E. J. Parish, R. A. Pascal, Jr., and A. A. Kandutsch. 1980. Inhibition of sterol biosynthesis in animal cells by 14α-hydroxymethyl sterols. *J. Lipid Res.* **21:** 571–584.
13. 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.
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. 1976. Mass spectrometry of sterols. Electron ionization induced fragmentation of C-4-alkylated cholesterols. *Chem. Phys. Lipids.* **17:** 466–500.
16. 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-15α-ol-3-one and 5α,14β-cholest-7-en-15β-ol-3-one. *Biochem. Pharmacol.* **28:** 249–252.
17. 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.
18. Zurcher, R. F., 1963. Protoneureson anz spektroskopic und Steroidstruktur II. *Helv. Chem. Acta.* **46:** 2054–2088.
19. Marker, R. E., E. L. Wittle, and L. W. Mixon. 1937. Sterols. XVI. Lanosterol and agnosterol. *J. Am. Chem. Soc.* **59:** 1368–1373.
20. Fieser, L. F., and M. Fieser. 1959. Steroids. Reinhold Publishing Company, New York, 365.
21. Gaylor, J. L. 1963. Biosynthesis of skin sterols. IV. Identification of labeled lanosta-7,24-dien-3β-ol from rat skin. *J. Biol. Chem.* **238:** 1649–1655.
22. 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.
23. Pascal, R. A., Jr., R. Shaw, and G. J. Schroepfer, Jr. 1979. Chemical synthesis of three 14α-hydroxymethyl cholestenols. *J. Lipid Res.* **20:** 570–578.
24. Brown, H. C., S. Ikegami, and J. H. Kawakami. 1970. Additions to bicyclic olefins. IV. The facile reduction of labile epoxides of bicyclic olefins by lithium in ethylenediamine. *J. Org. Chem.* **35:** 3243–3245.
25. Barton, D. H. R., and B. R. Thomas. 1953. Triterpenoids. Part XII. The lanosterol analogue of provitamin D_3. *J. Chem. Soc.* 1842–1847.
26. Fried, J., J. W. Brown, and M. Applebaum. 1965. Transformations of eburicoic acid. VI. 9α-Hydroxylanostane and eburicane derivatives and their photolytic fragmentation to 9,10-secosteroids. *Tetrahedron Lett.* 849–854.
27. Guest, I. G., and B. A. Marples. 1971. Steroids. Part XIV. Transformations of 8α,9α- and 9α,11α-epoxylanostanes and related compounds. *J. Chem. Soc. C.* 1468–1472.
28. Batten, P. L., T. J. Bentley, R. B. Boar, R. W. Draper, J. F. McGhie, and D. H. R. Barton. 1972. The synthesis of some 32-functionalized lanostane derivatives. *J. Chem. Soc. Perkin I.* 739–748.
29. 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-ene-3β,32-diol in cholesterol biosynthesis. *Bioorg. Chem.* **6:** 473–481.
30. Fried, J., and J. W. Brown. 1966. 32-Hydroxylated lanostane derivatives by photolysis of 3β-acetoxy-7-ketolanostane. *Tetrahedron Lett.* 1677–1680.
31. Bentley, T. J., J. F. McGhie, and D. H. R. Barton. 1965. The synthesis of 32-oxygenated lanostane derivatives. *Tetrahedon Lett.* 2497–2498.
32. Allen, J., R. B. Boar, and J. F. McGhie. 1973. Nitrite photolysis in the presence of oxygen. An improved synthesis of 32-oxygenated lanostanes. *J. Chem. Soc. Perkin I.* 2402–2404.
33. Barton, D. H. R., R. H. Hesse, M. M. Pechet, and L. C. Smith. 1979. The mechanism of the Barton reaction. *J. Chem. Soc. Perkin I.* 1159–1165.
34. Ruzicka, L., E. Rey, and A. C Muhr. 1944. 55. Zur Kenntnis der Triterpene. Uber verschiedene Umwandlungsprodukte des Lanosterins. *Helv. Chim. Acta.* **27:** 472–489.
35. Birchenough, M. J., and J. F. McGhie. 1950. Lanosterol. Part IX. Ketodihydrolanosterol. *J. Chem. Soc.* 1249–1252.
36. Barton, D. H. R., J. S. Fawcett, and B. R. Thomas. 1951. Triterpenoids. Part IV. Some observations on the constitution of lanostadienol (lanosterol). *J. Chem. Soc.* 3147–3154.
37. Shoppe, C. W., J. C. Coll, and R. E. Lack. 1968. Steroids. Part XXXI. Attempted modification of the 14α-methyl group in 4,4,14α-trimethyl steroids. *J. Chem. Soc. C.* 1581–1585.
38. Lassak, E. V., J. T. Pinhey, and J. J. H. Simes. 1973. The two C8 epimers of 3β-acetoxylanost-9(11)-en-7-one. *Aust. J. Chem.* **26:** 1051–1057.