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

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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 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 CHCl₃ 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.

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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 CDCl₃ 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 P₂O₅.

Commercially available “lanosterol” (ICN Pharmaceuticals, Inc., Cleveland, OH), after three recrystallizations from acetone–water to give a white crystalline solid (4.84 g), was found to be a mixture of lanosterol (48%) and 24,25-dihydrolanosterol (52%) upon GLC analysis (3% OV-17; 250°C).

Lanost-8-ene-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, the solvent was evaporated to dryness under reduced pressure and the resulting residue was recrystallized twice from aceton–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-ene-3β-ol (I) and lanost-7-ene-3β-ol (II)

A mixture of I and II was prepared by treatment of I with dry HCl gas in CHCl₃ 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 CHCl₃ (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 aceton–water (containing ~10% NH₄OH) and twice from aceton–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-ene-3β-ol (I) and lanost-7-ene-3β-ol (II)

To the mixture of I and II (5.00 g; 11.7 mmol) from above in CH₂Cl₂ (350 ml) were successively added NaHCO₃ (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 MgSO₄. The residue obtained upon evaporation of the solvent under reduced pressure was recrystallized from aceton–water to give a white crystalline solid (4.84 g).

TLC analyses in six solvent systems (SS-1, SS-2, SS-3, 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).

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
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 desiccator over P₂O₅ to give the crude product (5.05 g). Analysis by TLC (SS-1) showed two major components with Rf 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 x 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 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. The contents of fractions 31 through 41 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-CH₃; calc., 0.77), 0.88 (s, 3H, C-19-CH₃; calc., 0.91), 2.01 and 2.03 (s, 3H each, methyls of acetoxy functions), 4.53 (m, 1H, C-3-H); MS: 530 (3%; M⁺), 470 (34%; M - CH₃COOH), 455 (81%; M - CH₃ - CH₂COOH), 410 (21%; M - CH₃COOH - CH₂COOH), 395 (100%; M - CH₃ - CH₂COOH - CH₂COOH), 357 (4%; M - CH₃COOH - side chain), 330 (34%), 297 (9%; M - CH₃ - CH₂COOH - CH₂COOH), 270 (33%), and 255 (32%); [α]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-CH₃; calc., 0.77), 1.04 (s, 3H, C-19-CH₃; calc., 1.05), 1.99 (3H, methyl of acetoxy function), and 4.46 (m, 1H, C-3-H); MS: 488 (2%; M⁺), 470 (17%; M - H₂O), 455 (58%; M - CH₃ - H₂O), 428 (14%; M - CH₂COOH), 410 (6%; M - H₂O - CH₂COOH), 395 (54%; M - CH₃ - H₂O - CH₂COOH), 357 (3%; M - H₂O - side chain), 315 (4%; M - CH₂COOH - side chain), 297 (4%; M - H₂O - CH₂COOH - side chain), 291 (30%), 289 (13%), and 203 (40%); [α]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 (VII)

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 x 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-CH₃; 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,HCl); 315 (8%; M⁻CH₃COOH); 209.0–210.5°C (13), and 212°C (7); IR, 3440, 1744, and 1045 cm⁻¹; NMR, 0.76 (s, 3H, C-19-CH₃; calc., 0.76), 0.89 (s, 3H, C-19-CH₃; 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⁻H₂O), 455 (82%; M⁻CH₃-H₂O), 413 (3%; M⁻CH₃-CH₂COOH), 395 (100%; M⁻CH₂-H₂O-CH₂COOH), 380 (2%; C₅₋) and 375 (2%; M⁻H₂O-side chain), 315 (6%; M⁻CH₂COOH-side chain), and 297 (5%; M⁻H₂O-CH₂COOH-side chain); [α]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-1, SS-3, and SS-4). GLC analyses (3% OV-1 and 3% OV-17; 270°C) of the free sterol showed a purity of 98.8%.

**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 aluminium 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 saturated NH₄Cl solution and thoroughly extracted with ether containing CH₂Cl₂ 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-CH₃; calc., 0.77), 1.03 (s, 3H C-19-CH₃; calc., 1.03), and 3.19 (m, 1H, C-3-H); MS: 446 (2%; M⁺), 431 (2%; M⁻CH₃); 315 (2%; M⁻H₂O-side chain), 288 (70%), 273 (32%), and 262 (100%); [α]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%.

**3β-Acetoxy-7α,32-epoxy-lanostan-7α-ol (IX) from lanostane-3β,9α-diol (VI)**

To compound VI (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 MgSO₄ 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 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, 3480, 1744, and 1045 cm⁻¹; NMR, 0.76 (s, 3H, C-19-CH₃; calc., 0.76), 1.03 (s, 3H, C-19-CH₃; 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⁻H₂O), 455 (82%; M⁻CH₃-H₂O), 413 (3%; M⁻CH₃-CH₂COOH), 395 (100%; M⁻CH₂-H₂O-CH₂COOH), 380 (2%; C₅₋) and 375 (2%; M⁻H₂O-side chain), 315 (6%; M⁻CH₂COOH-side chain), and 297 (5%; M⁻H₂O-CH₂COOH-side chain); [α]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-1, SS-2), 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 98.8%.

**3β-Acetoxy-7α,32-epoxy-lanostan-7α-ol (IX) 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 MgSO₄ 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-CH₃), 0.86 (s, 3H, C-19-CH₃), 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⁻CH₃), 456 (74%; M⁻CH₂O), 455 (100%; M⁻CH₃-CH₂O), 441 (11%; M⁻CH₃-CH₂O), 413 (2% M⁻CH₂OH-42), 395 (55%; M⁻CH₃COOH

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-CH$_2$OH), 381 (14%; M - CH$_2$ - CH$_2$O - CH$_2$COOH), 375 (8%; M - side chain - 42), 313 (4%; M - side chain - CH$_3$COOH), and 283 (12%; M - CH$_2$O - side chain - CH$_3$COOH); [a]$_D$ + 25.9$^\circ$ (c, 0.47) (lit., +25$^\circ$ (7, 13) and +24$^\circ$ (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$_4$Cl solution and thoroughly extracted with ether containing CH$_2$Cl$_2$ (10%). The combined extracts were dried over anhydrous MgSO$_4$ and evaporated to dryness under reduced pressure. The resulting residue, after further drying over P$_2$O$_5$ 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$_4$Cl solution and thoroughly extracted with ether containing CH$_2$Cl$_2$ (10%). The combined extracts were dried over anhydrous MgSO$_4$ and evaporated to dryness under reduced pressure. The product showed a single component on TLC in three solvent systems (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; [a]$_D$ + 10.6$^\circ$ (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 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 = 8 Hz), 4.17 (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$_2$O), 414 (60%; M - CH$_2$O), 413 (100%; M - CH$_2$OH), 399 (12%; M - CH$_2$O - CH$_3$), 395 (33%; M - H$_2$O - CH$_2$OH), 331 (6%; M - CH$_2$O), 283 (12%; M - 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$_2$O), 485 (49%; M - CH$_2$OH), 471 (3%; M - CH$_2$O - CH$_2$O), 426 (5%; M - trimethylsilanol), 411 (2%; M - CH$_3$ - trimethylsilanol), 403 (3%; M - side chain), 396 (23%; M - CH$_2$O - trimethylsilanol), 395 (49%, M - CH$_3$OH - trimethylsilanol), 386 (60%), 381 (8%; M - CH$_3$ - CH$_2$O - trimethylsilanol), 298 (5%; M - CH$_3$OH - trimethylsilanol - side chain), and 283 (6%; M - CH$_2$O - 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$_2$O$_5$ 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$_4$Cl solution and thoroughly extracted with ether containing CH$_2$Cl$_2$ (10%). The combined extracts were dried over anhydrous MgSO$_4$ 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; [a]$_D$ + 10.6$^\circ$ (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 = 8 Hz), 4.17 (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$_2$O), 414 (60%; M - CH$_2$O), 413 (100%; M - CH$_2$OH), 399 (12%; M - CH$_2$O - CH$_3$), 395 (33%; M - H$_2$O - CH$_2$OH), 331 (6%; M - CH$_2$O), 283 (12%; M - 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$_2$O), 485 (49%; M - CH$_2$OH), 471 (3%; M - CH$_2$O - CH$_2$O), 426 (5%; M - trimethylsilanol), 411 (2%; M - CH$_3$ - trimethylsilanol), 403 (3%; M - side chain), 396 (23%; M - CH$_2$O - trimethylsilanol), 395 (49%, M - CH$_3$OH - trimethylsilanol), 386 (60%), 381 (8%; M - CH$_3$ - CH$_2$O - trimethylsilanol), 298 (5%; M - CH$_3$OH - trimethylsilanol - side chain), and 283 (6%; M - CH$_2$O - 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.
M – H₂O – CH₂O – side chain), and 259 (17%); MS on bis-TMS derivative: 588 (5%; M), 573 (8%; M – CH₃), 498 (54%; M – trimethylsilanol), 485 (19%; M – CH₂OSi(CH₃)₃), 408 (70%; M – trimethylsilanol – trimethylsilanol), 395 (100%; M – trimethylsilanol – CH₂OSi(CH₃)₂), 393 (56%; M – CH₃ – trimethylsilanol – trimethylsilanol), 285 (7%; M – trimethylsilanol – side chain), 367 (10%), 295 (20%; M – trimethylsilanol – trimethylsilanol – side chain); [α]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 (Rf, 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) 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 59 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-CH₃), calc., 0.76; 1.03 (s, C-19-CH₃), calc., 1.09; 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 – H₂O), 414 (100%; M – CH₂O), 413 (54%; M – CH₂OSi(CH₃)₂), 483 (6%; M – CH₃ – trimethylsilanol), 408 (2%; M – trimethylsilanol – trimethylsilanol), 395 (100%; M – trimethylsilanol – CH₂OSi(CH₃)₂), 393 (6%; M – CH₃ – trimethylsilanol – trimethylsilanol), 385 (19%; M – trimethylsilanol – side chain), 295 (25%; M – trimethylsilanol – trimethylsilanol – side chain), 241 (6%), and 227 (8%); [α]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,8-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 Δ⁸-steryl acetate, a complex mixture is obtained from which 3β-acetoxy-lanost-7-ene can be isolated (either directly or via oxidation of the proposed Δ⁷(8,11)-seryl intermediate) (19, 34–38). Reduction of the Δ⁸-7-keto-sterol 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-7-α-ol in 69% yield (28). 3β-Acetoxy-lanostan-7α-yl nitrite was prepared, in 92% yield, from the 7α-hydroxyster ery 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-nitro-lanostan-7α-yl methanesulfonate in 89% yield (28). The latter compound, upon heating with dry collidine, gave 3β-acetoxy-lanost-7-en-32-onitrite in 79% yield (28). Reduction of the nitrite 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-en-32,32-diol in 88% yield (28). Thus, by this approach, lanost-7-en-3β,32-diol can be obtained, in 11 steps, from 3β-acetoxy-lanost-8-ene. The yield of the Δ2-3β,32-diol from 3β-acetoxy-lanostan-7-one by this route is ~15%. The overall yield from the ultimate starting material (3β-acetoxy-lanostan-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-en-3β,32-diol by modifications of the approach outlined above were unsuccessful (28). This scheme was subsequently improved by the finding that photolyis 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-lanostan-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-en-3β,32-diol. The starting material for this synthesis was 3β-acetoxy-lanostan-7-one. This compound can be prepared from its corresponding Δ8-isomer (prepared from crude lanosterol obtained from lanolin (19)) by treatment with dry HCl in chloroform (19). Under these conditions, a mixture of the Δ8 and Δ7 isomers is obtained from which the Δ7-sterol acetate can be isolated, in unspecified yield, by chronic 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 Δ7-sterol acetate to the corresponding 7α,8α-epoxide, reduction of the latter compound with lithium in ethylenediamine 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 acetoxy cleavage of the 7α,32-oxide 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 acetolitic cleavage of the 7α,32-oxide yields not only the 32-oxygenated-Δ7-sterol derivative, but also products to which the Δ8 (7, 8) and Δ5 (8) iso- saeric 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-acetoxy methyl-5α-cholest-7-en-3β-ol (I) and its derivatives (10, 22, 23) 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-lanostan-7-en-32-al in 84% yield (32).

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and pyridine at room temperature to give, after MPLC, 3β-acetoxy-lanostan-9α-ol (VII) and 3β,7α,9α-triacetoxy-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-sterol 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-AgNO₃. 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 Δ⁶ (XII), Δ⁷ (XIII), and Δ⁸ (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 Δ⁶ and Δ⁸ 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.

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