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AN ALTERNATIVE SYNTHESIS OF 4,4-DIMETHYL-5α-CHOLESTA-8,14,24-TRIEN-3β-OL, AN INTERMEDIATE IN STEROL BIOSYNTHESIS AND A REPORTED ACTIVATOR OF MEIOSIS AND OF NUCLEAR ORPHAN RECEPTOR LXRα

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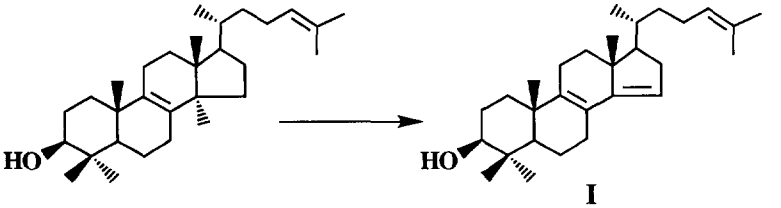
Abstract: 4,4-Dimethyl-5α-cholesta-8,14,24-trien-3β-ol, a sterol of current biological interest, has been synthesized in six steps from 3β-acetoxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one.

4,4-Dimethyl-5α-cholesta-8,14,24-trien-3β-ol, (**I**) is a product of the enzymatic 14α-demethylation of lanosterol.[1] Interest in **I** has been very considerably stimulated by reports that it activates meiosis in mammalian eggs,[2] that its formation from lanosterol in the rat ovary is under hormonal control,[3] and that it is a positive regulator of the nuclear orphan receptor LXRα.[4]



I

The availability of authentic **I** of known structure and high purity is critical to the pursuit of studies of these matters. The chemical synthesis of **I** requires the introduction of (1) the 4,4-dimethyl functionality, (2) the $\Delta^{8,14}$ diene system, and (3) the Δ^{24} olefinic bond. Variations in the order of introduction of these functionalities could be employed. However, consideration of readily available starting materials, e.g. cholesterol (readily available in high purity at relatively low cost) and ergosterol or various plant sterols (more costly and more difficult to obtain in a high state of purity) suggest synthetic routes in which the 4,4-dimethyl functionality is introduced first. Following this, a variety of approaches can be envisioned to permit introduction of the $\Delta^{8,14}$ and Δ^{24} olefinic systems.

In 1989, Dolle et al.[5] reported an eleven-step synthesis of **I** from the benzoate ester of 3β-hydroxy-4,4-dimethylergosta-8(14),22-dien-15-one. Their approach involved generation of the corresponding C-22 aldehyde by ozonolysis, elaboration of the desired C_8 side chain with oxygen functions at C-22 and C-24, removal of the oxygen functionality at C-22, introduction of the $\Delta^{8,14}$ diene system in the sterol nucleus, and finally, generation of the Δ^{24} double bond from the trifluoroacetate derivative of a $\Delta^{8,14}$-24-hydroxysterol. Whereas many intermediates were not isolated and characterized and the melting point of **I**

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was less than that reported herein, the overall synthesis was reported to proceed in high yield (~53%) from the 4,4-dimethyl-$\Delta^{8(14)}$-15-ketosteryl derivative of ergosterol.

We now report an alternative, relatively simple, six-step synthesis of **I** from 3β-acetoxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one[6a] (**II**) (Figure 1). A key step in the overall synthesis takes advantage of the highly efficient and specific oxidation of the saturated side chain of 3β-acetoxy-5α-cholest-8(14)-en-15-one with a mixture of trifluoroacetic anhydride, hydrogen peroxide, and sulfuric acid to give the C_{24} synthon, 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one.[7,8] Oxidation of **II** under these conditions provided the 4,4-dimethyl-$\Delta^{8(14)}$-15-keto-24-hydroxysteroid **III**[9] in notably high yield (74%). Swern oxidation of **III** gave, in 90% yield, the 24-aldehyde **IV**[10] which, upon Wittig olefination for construction of the Δ^{24} side chain produced the $\Delta^{8(14),24}$-15-ketosteroid **V**[11] in 75% yield. Borohydride reduction of **V** gave, after MPLC on silica gel, the 15β-hydroxysteroid **VI**[12] (81% yield) which, upon treatment with acid and MPLC on silica gel, provided the $\Delta^{8,14,24}$ steryl acetate **VII** in 85% yield.[13] Finally, saponification of **VII** under standard conditions gave the $\Delta^{8,14,24}$ free sterol **I**[14a] (100% yield).[15]

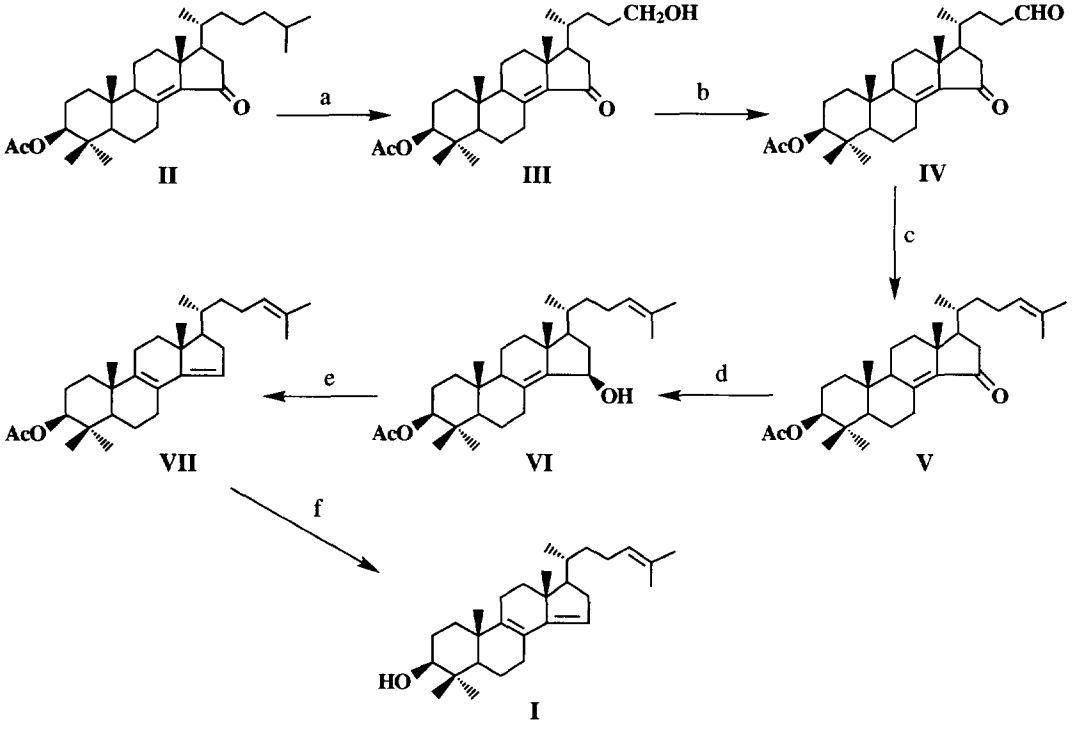

Figure 1. Conversion of 3β-acetoxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one (**II**) to 4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol (**I**): (a) $(CF_3CO)_2O$, H_2O_2, H_2SO_4, –2 °C; Na_2SO_3, K_2CO_3; (b) oxalyl chloride, dimethylsulfoxide, CH_2Cl_2, –50 °C, 1 h; Et_3N, 25 °C, 5 min; (c) isopropyltriphenylphosphonium iodide, butyllithium, THF, 0 °C, 2 h; (d) $NaBH_4$, EtOH, 25 °C; (e) H_2SO_4 (cat.), $CHCl_3$, 25 °C, 5 min; (f) KOH, EtOH, 70 °C, 2 h.

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References and Notes

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6. (a) Prepared from 3β-hydroxy-4,4-dimethyl-5α-cholest-8(14)-en-15-one[6b] by treatment with acetic anhydride and pyridine; M.P. 123.5-126 °C; single component on TLC and reversed-phase HPLC; MS, 470 (100%; M^+) calcd. for $C_{31}H_{50}O_3$ 470.3760, found 470.3760; IR, 1732, 1703, 1626 cm^{-1}; UV, λ_{max} (EtOH) 259 nm (ε 14,000); ^{13}C NMR, δ 80.68 (C-3), 150.08 (C-8), 140.00 (C-14), 208.23 (C-15); ^1H NMR, δ 0.800 (H-19), 0.969 (H-18), 0.994 (H-21).
   (b) Prepared by minor modifications of previously described method: Schroepfer, G. J., Jr.; Parish, E. J.; Tsuda, M.; Kandutsch, A. A. *Chem. Phys. Lipids* **1988,** *47*, 187.
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9. MP, 218–220 °C; single component on TLC and reversed-phase HPLC; MS, 444 (100%; M^+) calcd. for $C_{28}H_{44}O_4$ 444.3239, found 444.3247; IR, 3512, 1730, 1693, 1620 cm^{-1}; UV, λ_{max} (EtOH) 259 nm (ε 14,400); ^{13}C NMR, δ 80.68 (C-3), 150.33 (C-8), 139.84 (C-14), 207.90 (C-15), 63.27 (C-24); ^1H NMR, δ 0.801 (H-19), 0.977 (H-18), 1.020 (H-21).
10. MP, 199–200 °C; single component on TLC; MS, 442 (85%; M^+) calcd. for $C_{28}H_{42}O_4$ 442.3083, found 442.3086; IR, 1728, 1697, 1626 cm^{-1}; UV, λ_{max} (EtOH) 259 nm (ε 14,400); ^{13}C NMR, δ 80.63 (C-3), 150.56 (C-8), 139.60 (C-14), 207.29 (C-15), 202.33 (C-24); ^1H NMR, δ 0.801 (H-19), 0.978 (H-18), 1.004 (H-21).
11. MP, 128–129.5 °C; single component on TLC and reversed-phase HPLC; MS, 468 (100%; M^+) calcd. for $C_{31}H_{48}O_3$ 468.3603, found 468.3607; IR, 1732, 1703, 1694, 1622 cm^{-1}; UV, λ_{max} (EtOH) 259 nm (ε 14,700); ^{13}C NMR, δ 80.68 (C-3), 150.08 (C-8), 139.96 (C-14), 208.12 (C-15), 124.53 (C-24), 131.42 (C-25); ^1H NMR, δ 0.801 (H-19), 0.969 (H-18), 1.013 (H-21).

12. MP, 138.5–140 °C; single component on TLC and reversed-phase HPLC; MS, 470 (0.2%; M^+) calcd. for $C_{31}H_{50}O_3$ 470.3760, found 470.3751; ^{13}C NMR, δ 80.91 (C-3), 133.08 (C-8), 145.09 (C-14), 69.92 (C-15), 124.90 (C-24), 131.13 (C-25); ^1H NMR, δ 0.805 (H-19), 1.022 (H-18), 0.942 (H-21).

13. MP, 137–138 °C (lit. 126-128.5 °C[1b]; 139-140 °C[5]); MS, 452 (100%; M^+) calcd. for $C_{31}H_{48}O_2$ 452.3654, found 452.3653; IR 1736, 1640 cm^{-1}; ^{13}C NMR, δ 80.66 (C-3), 122.73 (C-8), 141.48 (C-9), 150.93 (C-14), 117.45 (C-15), 125.11 (C-24), 131.00 (C-25); ^1H NMR, δ 1.054 (H-19), 0.807 (H-18), 0.955 (H-21).

14. (a) Analytical sample obtained after Ag^+-HPLC[14b] and recrystallization: MP, 135–137 °C (lit. 119–121 °C[5]); single component on TLC and reversed-phase HPLC and purity of 99% on Ag^+-HPLC[14b] and 98–99% purity by ^1H NMR; MS, 410 (100%; M^+) calcd. for $C_{29}H_{46}O$ 410.3549, found 410.3555; UV, λ_{max} (EtOH) 249 nm (ε 18,600); ^{13}C NMR, δ 78.70 (C-3), 122.77 (C-8), 141.75 (C-9), 150.99 (C-14), 117.36 (C-15), 125.12 (C-24), 131.00 (C-25); ^1H NMR, δ 1.034 (H-19), 0.810 (H-18), 0.955 (H-21).

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15. It should be noted that this synthetic approach provides the basis for a relatively simple synthesis of isotopically substituted **I** in which the isotopic label is introduced via a suitably labeled Wittig reagent at a late stage in the synthesis of **I**.