STEREOSPECIFIC SYNTHESIS OF Δ^{24}-STEROLS LABELLED ON THE 26- OR 27-METHYL GROUP

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SUMMARY

A convenient synthesis of Δ^{24}-sterols labelled on the 26- or 27-methyl group, exemplified by the synthesis of [26-^{3}H]-lanosterol, is described. Oxidation of lanosteryl acetate with SeO_2 gives 3β-acetoxylanosta-8,24-dien-26-al; this is reduced to the corresponding labelled alcohol with KB^3H_4 and the obtained hydroxymethyl group is reduced to methyl group by hydrogenolysis of its sulfate monoester. 26-Hydroxylanosterol can be transformed into 27-hydroxy lanosterol by E ⟶ Z olefin inversion, so allowing the synthesis of lanosterol stereospecifically labelled at C-27.
Desmosteryl acetate analogously affords the corresponding 26-aldehyde, which is easily reduced to the 26-alcohol.

Key words: ^{3}H-stereospecific synthesis, lanosterol, desmosterol.

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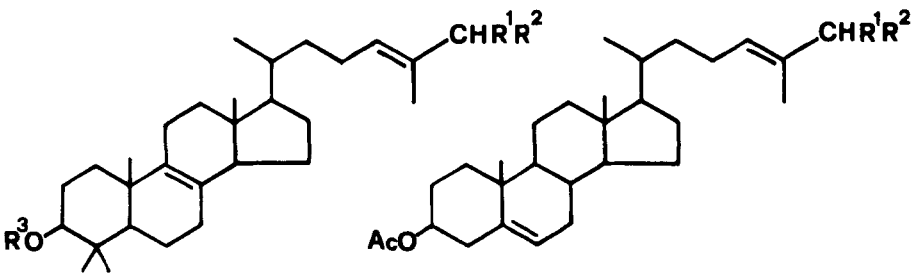
Some recent biosynthetic problems concern the stereochemistry at C-25 of several classes of steroidal compounds[1-4]. The solution of these problems requires the availability of Δ^{24} precursors of cholesterol, such as lanosterol or cycloartenol, stereospecifically labelled at C-26 or C-27. Such labelled compounds has been generally obtained by biosynthetic synthesis from suitably labelled mevalonic acid. This method presents several disadvantages, as the introduction of the label in other position of the molecule and the high cost and low specific activity of the precursor employed.

Bhalerao and Rapoport[5] have demonstrated the stereospecific oxidation of simple gem-dimethylolefins by SeO_2 to give E-aldehydes. We have utilized the same reaction to oxidize lanosteryl acetate (1) and desmosteryl acetate (10) to the corresponding aldehydes 2 and 11, the stereochemistry of which was assigned on the basis of the NMR signals[6] of the aldehydic hydrogen.



1 $R^1 = R^2 = H$; $R^3 = Ac$
2 $R^1, R^2 = O$; $R^3 = Ac$
3 $R^1 = H$; $R^2 = OH$; $R^3 = Ac$
4 $R^1 = {}^3H$; $R^2 = OH$; $R^3 = Ac$
5 $R^1 = H$; $R^2 = OTs$; $R^3 = Ac$
6 $R^1 = H$; $R^2 = OSO_3H$; $R^3 = Ac$
7 $R^1 = {}^3H$; $R^2 = OSO_3H$; $R^3 = Ac$
8 $R^1 = R^2 = R^3 = H$
9 $R^1 = {}^3H$; $R^2 = R^3 = H$

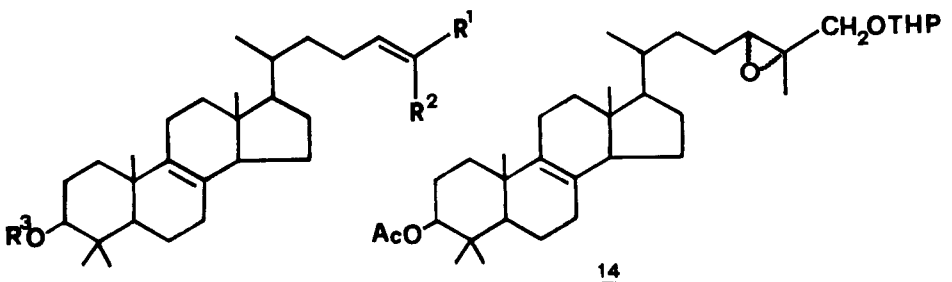
10 $R^1 = R^2 = H$
11 $R^1 R^2 = O$
12 $R^1 = H$; $R^2 = OH$

Reduction of 2 and 11 with $NaBH_4$ in 95% ethanol at room temperature, gave respectively the E-alcohols 3 and 12, the NMR signals of which confirmed the E stereochemistry of the Δ^{24} double bond[5].

The reduction of the primary allylic hydroxymethyl group of 3

to the methyl group of 8 gave some trouble; tosylation followed by $LiAlH_4$ hydrogenolysis of the tosylate 5 afforded a mixture of products containing only a small amount of lanosterol which could not be easily isolated in pure state, while good results were obtained working on the less reactive sulfate 6, which could be easily prepared from the alcohol 3 by treatment with SO_3-pyridine complex in tetrahydrofuran; reduction of this ester with a solution of $LiAlH_4$ in tetrahydrofuran[7] afforded good yields of nearly pure lanosterol, which was further purified through preparative tlc of the acetate on silicagel-$AgNO_3$. The same sequence of reactions was repeated using KB^3H_4 (25 mCi, 12 Ci/mM) for the reduction of 2 to yield 26-[^{3}H]-lanosterol (9) (2.2 mCi; 5.73×10^{11} dpm/mM).

The Z alcohol 16, necessary for the introduction of label into the 27-methyl group according to this method, was obtained by E→Z



14

13 $R^1=CH_2OTHP$; $R^2=CH_3$; $R^3=Ac$
15 $R^1=CH_3$; $R^2=CH_2OTHP$; $R^3=H$
16 $R^1=CH_2OH$; $R^2=CH_3$; $R^3=Ac$

isomerization of 3: the tetrahydropyranylderivative 13, obtained from 3 by acid-catalyzed exchange with 2-methoxytetrahydropyran, was epoxidized to 14 (as mixture of the stereoisomers) with m-chloroperbenzoic acid; the epoxides 14 were labile to Al_2O_3, but were easily purified by tlc on silicagel; treatment of these with lithium diphenylphosphide[8] followed by addition of excess of CH_3I, gave the Z-olefin 15, which was directly submitted to acetylation and then to hydrolysis of the tetrahydropyranyl protecting group to give the Z-allylic alcohol 16, the stereochemistry of which was con-

firmed by NMR data.

EXPERIMENTAL

Melting points are uncorrected; I.R. spectra were recorded in chloroform on a Perkin-Elmer 257 spectrophotometer. NMR spectra were obtained in deuteriochloroform on a Varian N.V. 14 spectrometer at 60 MHz using tetramethylsilane as reference. Optical rotations were recorded as 1% chloroform solutions on a Perkin-Elmer 141 polarimeter. Elemental analyses were consistent with the calculated values. Preparative and analytical thin layer chromatographies (tlc) were carried out on Merck HF_{254} silicagel plates; the products were detected under ultraviolet light and by spraying with aqueous sulfuric acid.

3β-Acetoxylanosta-8,24-dien-26-al (2). - A mixture of 15 g (32 mmol) of 3β-acetoxylanosta-8,24-diene (1) and 6.6 g (60 mmol) of SeO_2 in 830 ml of 95% ethanol was refluxed for 18 hr[5].

Removal of the solvent in vacuo, partition between ethyl ether and water and evaporation of the organic layer gave 18.1 g of crude oxidation product. This was chromatographed on silicagel-celite (1:1) and 3.4 g of the E-aldehyde (2) were eluted with benzene. Crystallization from ligroin afforded pure 2, m.p. 153-154°; $[\alpha]_D^{20}$= +54.9°; ν_{max} 1725, 1675 cm^{-1}. NMR: 0.70 (s, 18-CH_3), 0.88 (s, 28,29 and 30-CH_3's), 0.92 (d, J=6Hz, 21-CH_3), 1.01 (s, 19-CH_3), 1.76 (s, 27-CH_3), 2.04 (s, CH_3COO-), 4.53 (m, 3α-H), 6.50 (t, J=7Hz, -CH=C), 9.43 (s, -CHO) δ.

3β-Acetoxycholesta-5,24-dien-26-al (11). - 0.2 g of 3β-acetoxycholesta-5,24-diene (10) were oxidized with SeO_2 as above to yield 50 mg of the E-aldehyde 11, which was crystallized from ethyl ether: m.p. 131-133°; $[\alpha]_D^{20}$ = -41.9°; ν_{max} 1725, 1680 cm^{-1}. NMR: 0.68 (s,18-CH_3), 0.96 (d, J=6Hz, 21-CH_3), 1.00 (s, 19-CH_3), 1.74 (s, 27-CH_3), 2.00 (s, CH_3COO-), 4.60 (m, 3α-H), 5.36 (m, 6-H), 6.45 (t, J=7Hz, 24-H), 9.40 (s, -CHO) δ.

3β-Acetoxylanosta-8,24-dien-26-ol (3). - 0.7 g of $NaBH_4$ were added to a solution of 1.306 g of the aldehyde 2 into 140 ml of 95% ethanol. After stirring overnight at r.t., the excess of $NaBH_4$ was de-

stroyed with 1 N HCl. The ethanol was removed in vacuo and the product was extracted with chloroform. The organic layer was washed with H_2O and dried over Na_2SO_4; removal of the solvent in vacuo left a crude reaction product which was purified on silicagel-celite: elution with benzene yielded 0.964 g of the alcohol 3, which was crystallized from methanol-ethyl ether: m.p. 135°, $[\alpha]_D^{20}$ = + 53.2°; ν_{max} 3600, 1725 cm^{-1}. NMR: 0.70 (s, 18-CH_3), 0.88 (s, 28, 29 and 30-CH_3's), 0.91 (d, J=6Hz, 21-CH_3), 1.01 (s, 19-CH_3), 1.67 (s, 27-CH_3), 2.04 (s, CH_3COO-), 4.00 (s, CH_2OH), 4.55 (m,,3α-H), 5.41 (t, J=7 Hz, -CH=C) δ.

3β-Acetoxycholesta-5,24-dien-26-ol (12). - 50 mg of the aldehyde 11 were reduced as above to yield 35 mg of the alcohol 12 which was crystallized from isopropanol: m.p. 107-109°; $[\alpha]_D^{20}$ = -32.21°; ν_{max} 3600, 1725 cm^{-1}. NMR: 0.70 (s, 18-CH_3), 0.94 (d, J=6Hz, 21-CH_3), 1.03 (s, 19-CH_3), 1.68 (s, 27-CH_3), 2.02 (s, CH_3COO-), 4.00 (s, CH_2OH), 4.55 (m, 3α-H), 5.38 (m, 24-H and 6-H) δ.

Reduction of 3β-acetoxylanosta-8,24-dien-26-ol (3) to lanosta-8,24-dien-3β-ol (8). - 100 mg of pyridine-SO_3 complex[7] were added to a solution of 47 mg of 3β-acetoxylanosta-8,24-dien-26-ol (3) in 25 ml of tetrahydrofuran freshly distilled from $LiAlH_4$, and the mixture was stirred overnight at r.t.. After analysis by tlc (benzene-ethyl acetate 9:1) to control that the formation of the sulfate ester was completed, 25 ml of 0.01 N solution of $LiAlH_4$ in tetrahydrofuran were added at 0° to the stirred mixture. After 3 hr excess of $LiAlH_4$ was destroyed with 1 N HCl, tetrahydrofuran was removed in vacuo and the product extracted with ethyl ether. The organic layer was washed with H_2O, dried over Na_2SO_4 and the solvent removed in vacuo to give 40 mg of lanosta-8,24-dien-3β-ol (8), which was purified by acetylation and chromatography of the acetate on silicagel-$AgNO_3$ (20% $AgNO_3$); pentane-ethyl ether (30:1) eluted 38 mg of pure 3β-acetoxylanosta-8,24-diene (1), which was transformed into lanosta-8,24-dien-3β-ol (8) by reduction with $LiAlH_4$ as described before for the sulfate 6. The obtained compound was identical with an authentic sample of lanosta-8,24-dien-3β-ol.

3β-Acetoxy-26-p-toluenesulfonyllanosta-8,24-diene (5). - 50 mg of freshly recrystallized p-toluenesulfonyl chloride were added at 0° to a solution of 50 mg of 3β-acetoxylanosta-8,24-dien-26-ol (3) in

2 ml of dry pyridine. The mixture was kept overnight at 0°C and then poured into cold H_2O. Extraction with ethyl ether was followed by washing with dil. HCl and then with H_2O; the organic phase was dried over Na_2SO_4 and evaporated in vacuo to yield 52 mg of crude 5. This compound was directly reduced with $LiAlH_4$ as described above for the conversion of 6 to 8. Analysis by tlc of the obtained crude product showed a complex mixture of compounds in which only a small amount (~10%) of lanosta-8,24-dien-3β-ol (8) was present.

[26-^{3}H]-Lanosta-8,24-dien-3β-ol (9). - 5 mg of 3β-acetoxylanosta-8,24-dien-26-al (2) in 2 ml of 95% ethanol were stirred with KB^3H_4 (25 mCi, 12 Ci/mmol) for 4 hr at r.t.. 3 mg of cold KBH_4 were added to complete the reaction and after two additional hours the product was isolated as described for the alcohol 3 to give 5 mg (specific activity 1.36 x 10^9dpm/mg) of [26-^{3}H]-3β-acetoxylanosta-8,24-dien-26-ol (4), which were reduced as described above for the alcohol 3. The obtained crude product was purified byvacetylation and preparative tlc of the acetate on silicagel-$AgNO_3$ (20% $AgNO_3$), eluting with benzene-pentane 1:1. Reduction of the acetate afforded 3.6 mg (specific activity=1.34 x 10^9dpm/mg) of [26-^{3}H]-lanosta-8,24-dien-3β-ol (9), which was found to be chemically and radiochemically pure.

3β-Acetoxy-26-(2-tetrahydropyranyloxy)lanosta-8,24-dien (13). - 830 mg of 3β-acetoxylanosta-8,24-dien-26-ol (3) were dissolved into 13 ml of freshly distilled 2-methoxydihydropyran. 300 mg of Dowex 50 resin were then added and the mixture was stirred for 4 hr at 90° under nitrogen atmosphere. After cooling, the resin was separated by filtration and the filtrate was concentrated under reduced pressure, giving a residue which was chromatographed over Al_2O_3 II; benzene eluted 770 mg of the THP-ether 13, which was crystallized from methanol: m.p. 69-71°; ν_{max} 1725, 1125,1025 cm^{-1}.

3β-Acetoxy-26-(2-tetrahydropyranyloxy)-24,25-epoxylanost-8-ene (14). - 100 mg (50% excess) of m-chloroperbenzoic acid in 26 ml of chloroform were added dropwise with stirring to a cooled (0°C) solution of 220 mg of 3β-acetoxy-26-(2-tetrahydropyranyloxy)lanosta-8,24-diene (13) in 14 ml of chloroform. The mixture was then stirred for 3 additional hours at r.t. and the excess of m-chloroperbenzoic acid destroyed with 15 ml of 0.03 N $FeSO_4$. The organic layer was washed with 25 ml of $NaHCO_3$ and then with H_2O. Removal of the solvent in

vacuo afforded a crude product, which was purified by preparative tlc, using benzene-ethyl acetate 9;1 as eluant, to yield 130 mg of the epoxide 14, which was crystallized from methanol: m.p. 69-74°; ν_{max} 1725, 1125, 1025 cm^{-1}.

3β-Acetoxylanosta-8,24-dien-27-ol (16). - 10 ml of a 0.08 solution of lithium diphenylphosphide[8] in dry tetrahydrofuran were injected over a few minutes into a stirred solution of 95 mg of the epoxide 14 in 5 ml of dry tetrahydrofuran, under nitrogen atmosphere at r.t. . The resulting red solution was allowed to stand 30 minutes and then 0.3 ml of CH_3I were added. After 30 additional minutes, an analytical tlc using benzene-ethyl acetate as eluant, showed the complete formation of the 26-tetrahydropyranyloxylanosta-8,24-dien-3β-ol (15). This compound was recovered by addition of 5 ml of H_2O to the reaction mixture, removal of tetrahydrofuran in vacuo, extraction with ethyl ether and evaporation of the organic layer. The crude product was acetylated with 0.1 ml of Ac_2O in 5 ml of dry pyridine; usual work-up afforded 80 mg of crude acetate, which was dissolved in 10 ml of methanol and treated with 5 mg of p-toluenesulfonic acid overnight with stirring at r.t.. Purification of the crude product by preparative tlc (2 elutions with benzene-ethyl acetate 9:1) yielded 45 mg of 3β-acetoxylanosta-8,24-dien-27-ol (16) which was crystallized from ethanol: m.p. 143-146°, $[\alpha]_D^{20}$=+52.04°; ν_{max} 3600, 1725 cm^{-1}. NMR: 0.70 (s, 18-CH_3), 0.88 (s, 28,29 and 30-CH_3's), 0.92 (d, J=6Hz, 21-CH_3), 1.01 (s, 19-CH_3), 1.80 (s, 26-CH_3), 2.04 (s, CH_3COO-), 4.17 (s, CH_2OH), 4.55 (m, 3α-H), 5.38 (t, J=7Hz, -CH=C).δ.

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