SYNTHESIS OF (25S)-5α-CHOLESTANE-3β,26-DIOL[2,4,2',4'-$^3H_4$]

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SUMMARY

(25S)-5α-cholestane-3β,26-diol [2,4,2',4'-$^3H_4$] was synthesized by hydrogenation of neotigogenin acetate 2, followed by acetylation to (25S)-5α-furostane-3β, 26-diol diacetate 5; this was oxidized to (25S)-16,22--dioxo-5α-cholestane-3β,26-diol diacetate 6. Clemmensen reduction of the last product afforded (25S)-5α--cholestane-3β,26-diol 26-monoacetate 9, which was oxidized to 3-oxo-derivative 12; this was tritium labelled by base-catalyzed exchange with 0.1 N-NaOH in iso PrO^{3}H and reduced to 14 with $NaBH_4$.

Key Words: (25S)-5α-cholestane-3β,26-diol [2,4,2',4'-$^3H_4$], biosynthesis, neotigogenin, tomatidine.

The details of the biosynthetic pathway leading to steroidal sapogenins and spirosolanes are less known for the members with (25S)-configuration than for the (25R)-analogues. This may also be due to the fact that the biosynthetic intermediates of the (25S)--series are less accessibles.

In a study (1) of the biosynthesis of neotigogenin 1 and tomatidine 3 we needed labelled (25S)-5α-cholestane-3β,26-diol, which was synthesized as follow: neotigogenin 1, small quantities of which are contained in commercial tigogenin, was separated from this com

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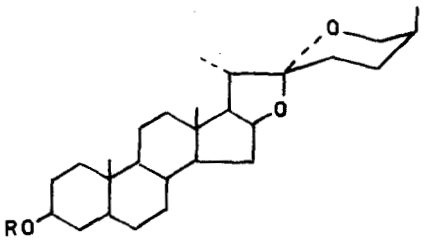
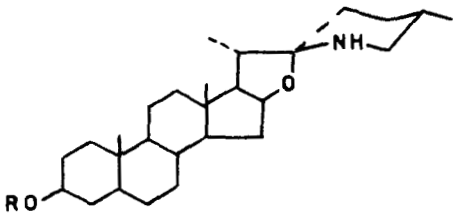


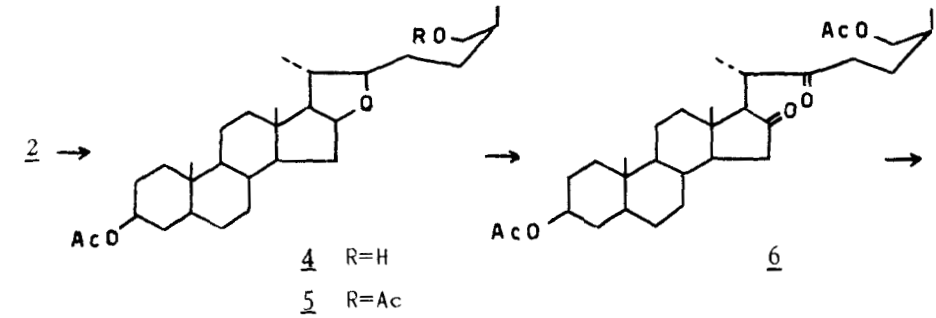
1 R=H
2 R=Ac



3 R=H

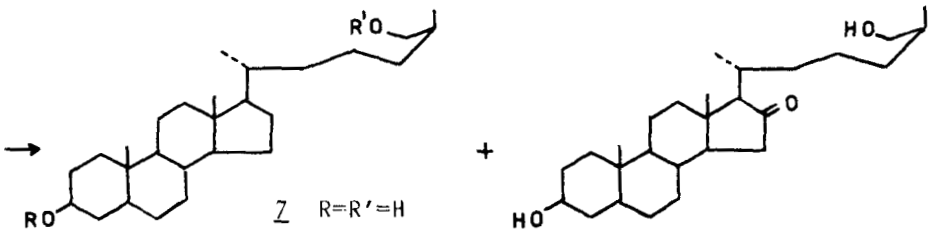
pound by fractional cristallization of the acetates and careful column chromatography of the mother liquors.

Catalytic hydrogenation of neotigogenin acetate 2 gave (25S)-5α--furostane-3β,26-diol 3-monoacetate 4, which was acetylated to 5; this diacetate was oxidized to (25S)-16,22-dioxo-5α-cholestane-3β, 26-diol diacetate 6 with CrO_3 in acetic acid.



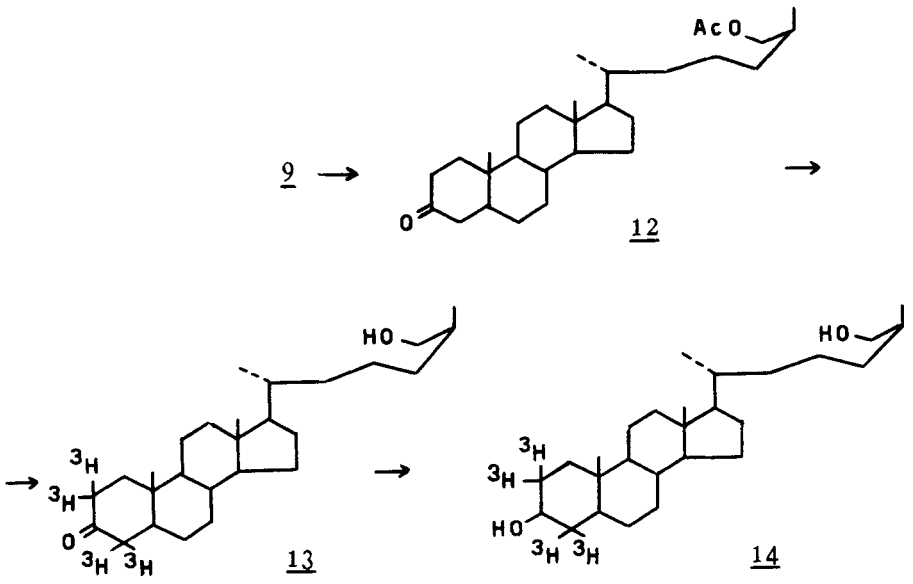
4 R=H
5 R=Ac

6



7 R=R'=H
8 R=Ac R'=H
9 R=H R'=Ac
10 R=Ac R'=Ac

11



Clemmensen reduction of the last product converted it to (25S)--5α-cholestane-3β,26-diol 7 and to (25S)-3β,26-dihydroxy-5α-cholestan-16-one 11; acetylation of 7 with 1 molar equivalent of acetic anhydride in pyridine afforded the 3-monoacetate 8 along with unreacted 7, 26-monoacetate 9 and diacetate 10. The monoacetate 8 was tosylated and reduced with $LiAlH_4$; the product obtained was purified and found to be identical to a known cholestan-3β-ol; this demonstrated that the above reactions did not alter the configurations at the chiral centres. The 26-monoacetate 9 was oxidized to 3-oxo-derivative 12, which was tritium-labelled 13 by base-catalyzed exchange with 0.1 N-NaOH in iso PrO^3H and reduced with $NaBH_4$.

Purification with preparative TLC afforded (25S)-5α-cholestane--3β,26-diol-[2,4,2',4'-$^3H_4$] 14 with a specific activity of 61.7 mCi/mMole.

The 25S configuration of the above compounds was confirmed by the M_D values, which showed a negative contribution of the (25S)--26-hydroxyl group (mean value obtained from 7, 8, 13 = -23.7°), which is in agreement with the data obtained by R. Tschesche (2).

EXPERIMENTAL SECTION

Melting points were determined on a Kofler hot-plate microscope and are uncorrected. Infrared (ir) spectra were recorded with a Perkin-Elmer 237 spectrophotometer. Nuclear magnetic resonance (nmr) spectra were recorded with a Perkin-Elmer R12 spectrometer at 60 MHz using tetramethylsilane as internal standard. The specific rotations were measured with a Perkin-Elmer 141 polarimeter.

Neotigogenin acetate 2

Neotigogenin acetate was obtained from the mother liquors after cristallization of commercial tigogenin acetate by column chromatography on silica gel-Celite 535 (1:1). Petroleum ether-methylene chloride (7:3) eluted 9.35 g of pure neotigogenin acetate from 200 g of commercial tigogenin; mp 175-179°C; $[\alpha]_D^{25}$-73.1° (c 0.32, $CHCl_3$) [Cf. (3): mp 174-176°C; $[\alpha]_D^{25}$-73.4° ($CHCl_3$)]; analysis calcd. for $C_{29}H_{46}O_4$: C 75.94, H 10.11, found: C 77.06, H 9.82. The ir spectrum presents the typical pattern of (25S)-sapogenins (986, 920, 900, 850 cm^{-1}) (4) and that of the deacetylated derivative is identical to that published by H. Sato (5).

(25S)-5α-furostane-3β,26-diol 3-monoacetate 4

9 g of neotigogenin acetate was hydrogenated as described (6), obtaining 8.8 g of 4; mp 108-110°C [Cf. (7): 107-111°C]; $[\alpha]_D^{25}$-15° (c 0.22, $CHCl_3$); ir (KBr) 3460, 3370, 1735, 1240 cm^{-1}.

(25S)-16,22-dioxo-5α-cholestane-3β,26-diol diacetate 6

8.7 g of 4 was acetylated with acetic anhydride-pyridine to (25S)-5α-furostane-3β,26-diol diacetate 5 (9.3 g of oily product), which was oxidized with CrO_3 in acetic acid (8). The crude (25S)--16,22-dioxo-5α-cholestane-3β,26-diol diacetate 6 was cristallized from methanol-water (7:3) to yield 6 g of pure compound, mp 114--118°C; $[\alpha]_D^{25}$-122.5° (c 0.29, $CHCl_3$); ir (KBr) 1735, 1720, 1250 cm^{-1}; analysis calcd. for $C_{31}H_{48}O_6$: C 72.06, H 9.36, found: C 72.54, H 9.56; nmr ($CDCl_3$): δ 0.78 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3), 0.92 (d J=7 Hz, 3H, 27-CH_3), 0.96 (d J=7 Hz, 3H, 21-CH_3), 2.00 (s, 3H, $\underline{CH_3}COOR$), 2.03 (s, 3H, $\underline{CH_3}COOR$), 2.6 (m, 2H, $\underline{CH_2}COR$), 3.93 (d,

J=6 Hz, 2H, $\underline{CH_2}OCOR$), 4.5-4.8 (m, 1H, 3-$\underline{C}$HOAc).

(25S)-5α-cholestane-3β,26-diol 7 and (25S)-3β,26-dihydroxy-5α-cholestan-16-one 11

Clemmensen reduction (9) of 5 g of 6 yielded 4.2 g of crude compound which was chromatographed on silica gel-Celite 535 (1:1) and eluted with petroleum ether-acetone (9:1); 2.2 g of (25S)-5α-cholestane-3β,26-diol 7 and 1 g of (25S)-3β,26-dihydroxy-5α-cholestan-16-one 11 were obtained.

The diol 7 had mp 167-172°C; $[\alpha]_D^{25}$+17° (c 0.34, $CHCl_3$); ir (KBr) 3250 cm^{-1}; analysis calcd. for $C_{27}H_{48}O_2$: C 80.14, H 11.96, found: C 80.62, H 11.78; nmr ($CDCl_3$) δ 0.64 (s, 3H, 18-CH_3), 0.80 (s, 3H, 19-CH_3), 0.87 (d, 3H, 27-CH_3), 0.90 (d, 3H, 21-CH_3), 3.45 (d J=6 Hz, 2H, 26-$\underline{CH_2}$OH), 3.4-3.7 (m, 1H, 3-CH).

The ketone 11 had mp 149-151°C; analysis calcd. for $C_{27}H_{46}O_3$: C 77.46, H 11.08, found: C 78.16, H 10.88; ir (KBr): 3300 (broad), 1740 cm^{-1}.

Acetylation of (25S)-5α-cholestane-3β,26-diol

The diol 7 was acetylated with Ac_2O (1 molar equivalent) in pyridine at rt to give a mixture of unreacted 3,26-diol 7, 3-monoacetate 8, 26-monoacetate 9 and 3,26-diacetate 10.

These products were isolated by chromatography on silica gel-Celite 535 (1:1) by elution with benzene-ethyl acetate (9:1).

(25S)-5α-cholestane-3β,26-diol 3-monoacetate 8

This compound had mp 125-127°C, $[\alpha]_D^{25}$+8° (c 1.68, $CHCl_3$); analysis calcd. for $C_{29}H_{50}O_3$: C 77.97, H 11.28, found: C 78.46, H 11.11; ir (KBr) broad between 3100-3050; nmr ($CDCl_3$): δ 0.65 (s,3H,18-CH_3), 0.82 (s, 3H, 19-CH_3), 0.87 (d, 3H, 27-CH_3), 0.92 (d, 3H, 21-CH_3), 2.00 (s, 3H, CH_3COOR), 3.45 (d J=6 Hz, 2H, 26-$\underline{CH_2}$OH), 4.5-4.8 (m, 1H, 3-CH).

(25S)-5α-cholestane-3β,26-diol 26-monoacetate 9

This product had mp 78-85°C; $[\alpha]_D^{25}$+20° (c 0.38, $CHCl_3$), analysis calcd. for $C_{29}H_{50}O_3$: C 77.97, H 11.28, found: C 78.40,H 10.92;

ir (KBr): broad between 3600-3200, 1740, 1240 cm^{-1}; nmr ($CDCl_3$) δ 0.62 (s, 3H, 18-CH_3), 0.79 (s, 3H, 19-CH_3), 0.87 (d, 3H, 27-CH_3), 0.92 (d, 3H, 21-CH_3), 2.03 (s, 3H, CH_3COOR), 3.4-3.7 (m, 1H, 3-CH), 3.90 (d, 2H, 26-$\underline{CH_2}OCOCH_3$).

(25S)-5α-cholestane-3β,26-diol diacetate 10

This compound had mp 108-112°C; $[\alpha]_D^{25}$+10° (c 0.53, $CHCl_3$); analysis calcd. for $C_{31}H_{52}O_4$: C 76.18, H 10.72, found: C 76.61, H 10.51; ir (KBr): 1745, 1250 cm^{-1}; nmr ($CDCl_3$) δ 0.65 (s, 3H, 18-CH_3), 0.82 (s, 3H, 19-CH_3), 0.87 (d, 3H, 27-CH_3), 0.92 (d, 3H, 21-CH_3), 2.00 (s, 3H, CH_3COOR), 2.03 (s, 3H, CH_3COOR), 3.90 (d, 2H, 26-$\underline{CH_2}OAc$), 4.5-4.8 (m, 1H, 3-CH).

(25S)-3-oxo-5α-cholestan-26-yl acetate 12

0.5 g of (25S)-5α-cholestane-3β,26-diol 26-monoacetate 9 was oxidized with CrO_3 by Jones' procedure (10) to (25S)-3-oxo-5α-cholestan-26-yl 12 (0.48 g) of oily product, chromatographically pure: ir (nujol) 1745, 1720, 1240 cm^{-1}; nmr ($CDCl_3$) δ 0.66 (s, 3H, 18-CH_3), 0.84 (s, 3H, 19-CH_3), 2.02 (s, 3H, CH_3COOR), 3.86 (d, J=7 Hz, 2H $\underline{CH_2}OCOCH_3$).

Hydrolysis of 12 with KOH in methanol, gave (25S)-26-hydroxy-5α-cholestan-3-one, which was crystallized from methanol: mp 136-138°C; $[\alpha]_D^{25}$+33° (c 0.34, $CHCl_3$); analysis calcd. for $C_{27}H_{46}O_2$: C 80.54, H 11.52, found: C 81.07, H 11.33; ir ($CHCl_3$): 3620, 1710 cm^{-1}; nmr ($CDCl_3$) 0.75 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 3.51 (d, J=6 Hz, 2H, $\underline{CH_2}OH$).

(25S)-5α-cholestane-3β,26-diol-[2,4,2',4'-$^3H_4$] 14

(25S)-3-oxo-5α-cholestan-26-yl acetate 12 (15 mg) was dissolved in 7.6 ml of 0.1 N NaOH in iso-PrO^3H (2 Ci) and refluxed under N_2 for 5 h. The solution was lyophilized and the solid residue redissolved in $CHCl_3$; the solution was washed with H_2O, dried over Na_2SO_4 and evaporated in vacuo. The residue 13 was reduced with $NaBH_4$ in ethanol to yield (25S)-5α-cholestane-3β,26-diol-[2,4,2',4'-$^3H_4$] 14.

Purification by preparative TLC with benzene-ethyl acetate (9:1) sis calcd. for $C_{29}H_{50}O_3$: C 77.97, H 11.28, found: C 78.40, H 10.92; ir (KBr): broad between 3600-3200, 1740, 1240 cm^{-1}; nmr ($CDCl_3$) δ

0.62 (s, 3H, 18-CH_3), 0.79 (s, 3H, 19-CH_3), 0.87 (d, 3H, 27-CH_3), 0.92 (d, 3H, 21-CH_3), 2.03 (s, 3H, CH_3COOR), 3.4-3.7 (m, 1H, 3-CH), 3.90 (d, 2H, 26-$\underline{CH_2}OCOCH_3$).

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(25S)-3-oxo-5α-cholestan-26-yl acetate 12

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(25S)-5α-cholestane-3β,26-diol-[2,4,2',4'-$^3H_4$] 14

(25S)-3-oxo-5α-cholestan-26-yl acetate 12 (10 mg) was dissolved in 0.1 N NaOH in iso-PrO^3H and refluxed under N_2 for 5 h. The solvent was removed in vacuo at rt and the solid residue redissolved in $CHCl_3$; the solution was washed with H_2O, dried over Na_2SO_4 and evaporated in vacuo. The residue 13 was reduced with NaB^3H_4 in ethanol to yeld (25S)-5α-cholestane-3β,26-diol-[2,4,2',4'-$^3H_4$] 14.

After purification using preparative tlc with benzene-ethyl ace afforded 11 mg of chromatographically pure 14, with a specific activity of 61.7 mC/mMole.

The above compound appeared to be chemically and radiochemically homogeneous also by TLC with petroleum ether-acetone (7:3) and chloroform-methanol (95:5).

The location of the label at the 2,4,2',4' positions was demonstrated by chromic acid oxidation of 14 followed by back-exchange of the crude compound with unlabelled 0.1 N NaOH in iso-PrOH and the result was the complete loss of tritium.

The labelled compound 14 is stable for at least one month; in fact it was administred as a biogenetic precursor to Lycopersicon Pimpinellifolium plants in a one month period, during which TLC controls showed that it was chemically and radiochemically unaltered.

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