Spirostanic analogues of teasterone. Synthesis, characterisation and biological activity of laxogenin, (23*S*)-hydroxylaxogenin and 23-ketolaxogenin (23-oxolaxogenin)

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Received (in Cambridge, UK) 20th September 2000, Accepted 10th November 2000
First published as an Advance Article on the web 16th January 2001

The synthesis and characterisation of the naturally occurring steroid sapogenin laxogenin **1** and its derivatives 23-ketolaxogenin **10** and (23*S*)-hydroxylaxogenin **13** are described. Compounds reported have shown plant-growth-stimulating activity in *in vitro* tests and in field trials.

Introduction

Brassinosteroids represent a new class of phytohormones, which produce a potent plant-growth-stimulating effect.[1] A number of synthetic analogues bearing structural modifications at the side chain and/or the steroidal skeleton have shown properties as plant-growth regulators.[2,3] As part of our screening programme we have directed our attention to the synthesis of different steroids with 5β-cholanic,[4,5] furostanic,[6–8] and spirostanic[9–12] skeletons which bear the characteristic functionality of some naturally occurring brassinosteroids.

Spirostanic sapogenins are widespread in the plant kingdom. Laxogenin or (25*R*)-3β-hydroxy-5α-spirostan-6-one **1** isolated from *Smilax sieboldi*[13] is, to the best of our knowledge, one of only three steroidal sapogenins which bear a carbonyl function at position C-6 (see Fig. 1). This characteristic, together with the β-hydroxy group at position C-3, makes laxogenin ***a naturally occurring spirostanic analogue of the naturally occurring brassinosteroid teasterone 2***. We now report on the synthesis, characterisation and biological activity of laxogenin **1**, 23-ketolaxogenin **10** and (23*S*)-hydroxylaxogenin **13**.

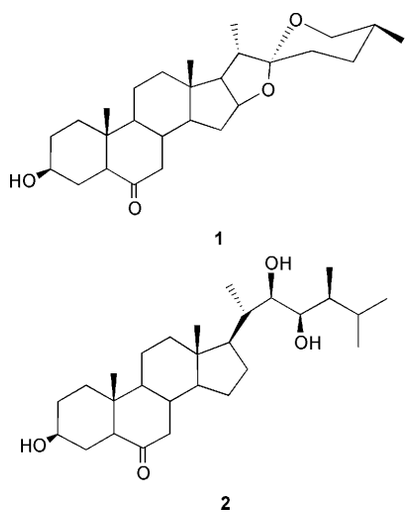


Fig. 1 The naturally occurring steroids laxogenin **1** and teasterone **2**.

Results and discussion

Brown's hydroboration of diosgenin **3** followed by oxidative replacement of the organoborane with hydrogen peroxide in basic media led to the naturally occurring chlorogenin **4**, which was submitted without purification to Jones oxidation to produce the dione **5** (Scheme 1). Catalytic hydrogenation over PtO_2 or reduction with $NaBH_4$ of the dione **5** produced exclusively the 3β,6β-diol **6** (β-chlorogenin), which was oxidised with *N*-bromosuccinimide (NBS)[14] in acetone to the naturally occurring laxogenin **1**.

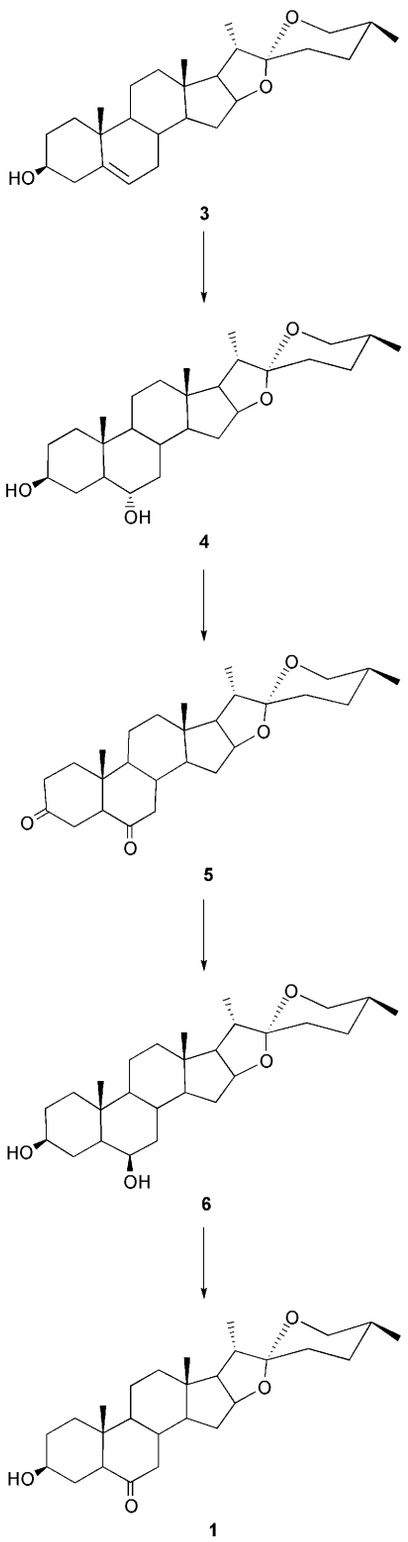
Acetylation of the diol **6** produced the diacetate **7**, which on treatment with $NaNO_2$ in acetic acid as previously described[10,12] led to the 23-keto compound **8** (Scheme 2). Saponification to the dihydroxylated ketone **9** followed by selective oxidation of the 6β-hydroxy function with NBS afforded 23-ketolaxogenin **10**.

Reduction, with $NaBH_4$, of the diacetylated ketone **8** mainly produced the (23*S*)-hydroxy compound **11**, which on saponification and selective oxidation of the 6β-hydroxy function with NBS afforded (23*S*)-hydroxylaxogenin **13** (Scheme 3). Acetylation, followed by selective saponification of the 3β-acetate, led to the 23-monoacetate of (23*S*)-23-hydroxylaxogenin compound **15**.

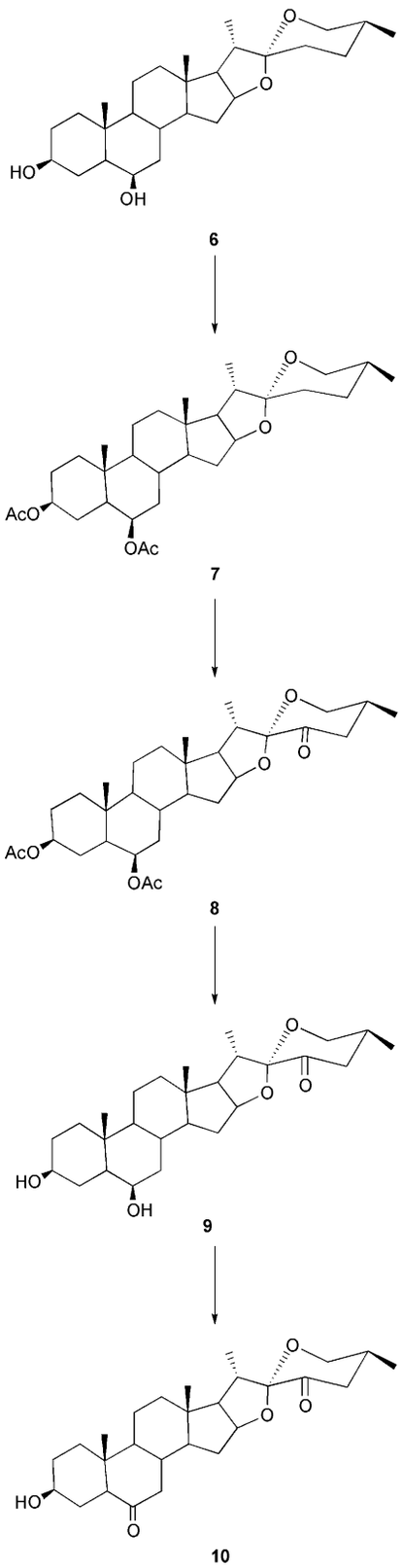
The structures of the hitherto unknown compounds are supported by the spectral data obtained. ^1H and ^{13}C NMR chemical shifts of the nuclei in rings A, B and C are in good agreement with the shielding data available for equivalent substitution patterns at the steroid skeleton[14,15] or the side chain.[16] The configuration at C-23 in the 23-hydroxylated compounds could be easily determined after acetylation. Coupling constants of 23-H (dd, *J* 11.8 and *J* 4.8 Hz) indicate its axial orientation and consequently the *S* configuration for the stereogenic centre C-23 (see Fig. 2). A detailed discussion on the ^{13}C and ^1H spectral characteristics derived from the introduction of oxygenated substituents at position C-23 of spirostanic sapogenins can be found in ref. 16.

Solutions of laxogenin **1**, 23-ketolaxogenin **10** and (23*S*)-23-hydroxylaxogenin **13** (concentrations 0.1 and 0.01 ppm) showed strong plant-growth-promoting activity in the radish test.[18] The effect consisted of an increase of the length of the treated hypocotyls compared with that of the non-treated plants (see Fig. 3). Besides, when the same solutions were applied to seeds of orange tree, a strong promotion of the germination was observed. The effect consists of an increase of the number of germinated seeds. Stronger promoting effects were observed for the C-23 oxygenated analogues.

DOI: 10.1039/b007656m



Scheme 1



Scheme 2

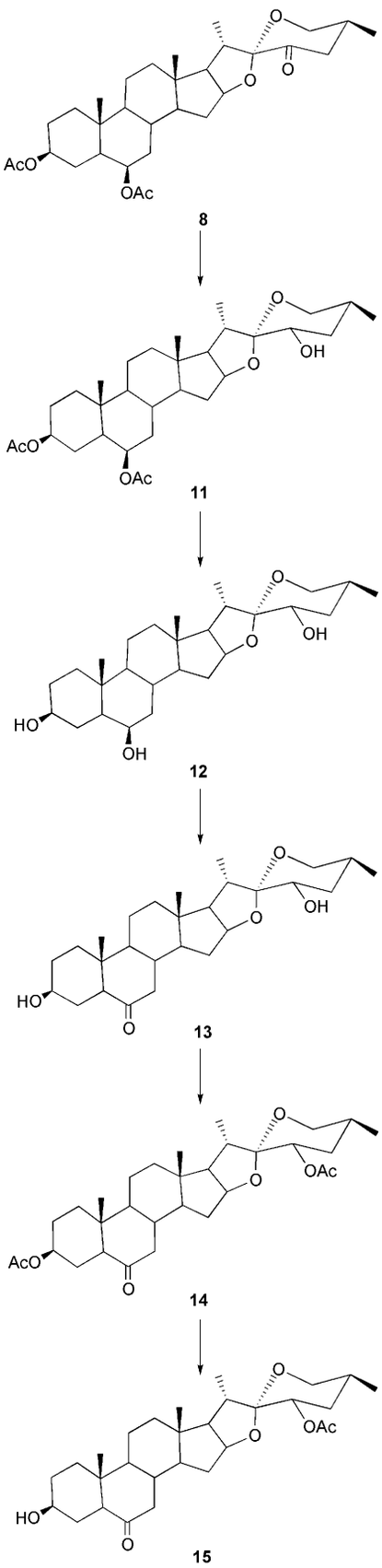
The spirostanic skeleton is a very common structure in plant tissues. Given the facts that laxogenin **1** showed growth activity and that the introduction of oxygenated functions at C-23 results in an increase in the biological activity, two alternatives should be considered.

(a) *The compound itself is active.* Then, the magnitude of the biological activity will depend on how well the analogue fits with the brassinosteroid receptor. As a consequence of the limited mobility of the side chain, spirostanic analogues of brassinosteroids may be useful for quantitative structure–activity relationship (QSAR) studies.

(b) *The compound is transformed into an active form.* This could mean that laxogenin could take part in the biosynthesis of teasterone **2**.

Conclusions

We have designed synthetic sequences that allow the preparation of spirostanic analogues of the brassinosteroid teasterone **2** from the readily available steroidal sapogenin diosgenin **3**.



Scheme 3

Compounds reported have shown a potent plant-growth-stimulating activity; introduction of an oxygenated function at position C-23 produces an increase in the plant-growth-promoting effect. Further biological experiments to compare the biological activity of the reported compounds with that of the naturally occurring brassinosteroids are required.

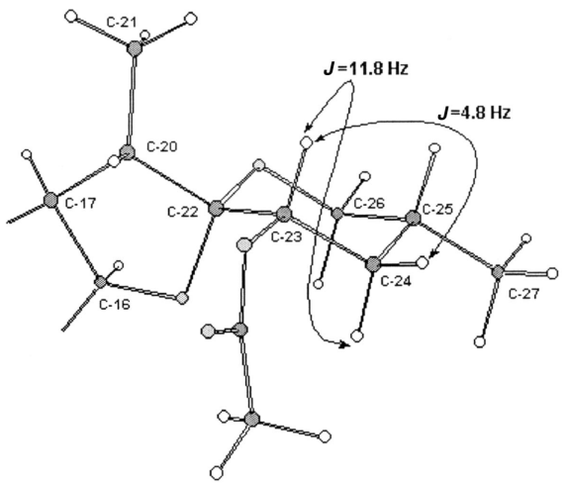


Fig. 2 MM+-Optimised geometry[17] of the side chain of a 23-acetoxy compound.

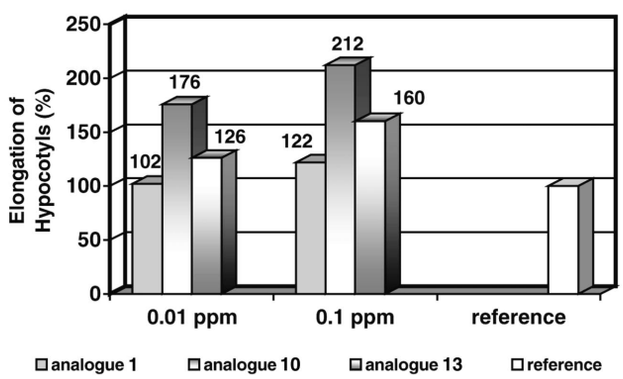


Fig. 3 Result of radish test (reference non-treated plants).

Experimental

IR spectra were recorded on KBr cells on a Philips Analytical PU9800 FT-IR spectrometer. NMR spectra were recorded on a Bruker 250 ACF spectrometer using $CDCl_3$ as solvent with $SiMe_4$ (TMS) (^1H) or the solvent signal at δ_C 77.0 (^{13}C) as reference. The temperature of the probes was 300 K and concentration rates were 30 mg mL^{-1} for ^{13}C spectra and 10 mg mL^{-1} for ^1H spectra. *J*-Values are given in Hz. Mps were measured on Electrothermal 9100 equipment and are uncorrected.

(25*R*)-5α-Spirostan-3,6-dione 5

$BF_3{\cdot}Et_2O$ (3.2 mL) was added dropwise (1 h) to a stirred solution of diosgenin **3** (10 g, 24 mmol) and $NaBH_4$ (10 g, 260 mmol) in dry THF (150 mL) and the resulting mixture was stirred for 24 h before cooling to 10 °C and addition of saturated aq. NaCl (200 mL). The solid produced was filtered off with suction, dried in air, dissolved in a solution of KOH/methanol (5 g/250 mL) and 35% aq. H_2O_2 (25 mL) was added over a period of 1 h. The mixture was poured into water (300 mL) and the solid was filtered off with suction, dried at 60 °C, and dissolved in acetone (150 mL). An excess of Jones reagent was added in small portions to the stirred and cooled (10 °C) solution. Stirring was continued for a further 15 min before the addition of propan-2-ol (10 mL). The mixture was poured into water, and the solid was filtered off, washed with water, dried at 60 °C, and purified by column chromatography on silica gel (heptane–ethyl acetate 95:5 to 80:20) to afford 7.9 g (77%) of the dione **5**, mp 243.6–244.5 °C (from acetone/*n*-heptane) (Calc. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.31; H, 9.50%); IR (ν_{max}/cm^{-1}) 2953 and 2910 (νCsp^3-H),

1719 (νC=O), 1452 and 1381 (δCH), 1090, 1059 and 1044 (νC-O), 981, 920, 900 and 870 (spiroketal); ^{1}H NMR (δ) 0.79 (3 H, d, J 6.3, H_3-27), 0.81 (3 H, s, H_3-18), 0.98 (3 H, s, H_3-19), 0.98 (3 H, d, J 6.7, H_3-21), 3.35 (t, J 10.7, 26-H_{ax}), 3.44 (ddd, J 1.9 and J 4.9 and J 10.4, 26-H_{eq}), 4.44 (1 H, m, 16-H); ^{13}C NMR (δ) C-1 37.8, C-2 37.2, C-3 210.7, C-4 36.8, C-5 57.2, C-6 208.5, C-7 46.4, C-8 37.2, C-9 53.2, C-10 41.0, C-11 21.3, C-12 39.2, C-13 40.8, C-14 56.1, C-15 31.2, C-16 80.2, C-17 61.9, C-18 16.2, C-19 12.4, C-20 41.4, C-21 14.3, C-22 109.1, C-23 31.4, C-24 28.6, C-25 30.1, C-26 66.7, C-27 17.1.

(25*R*)-5α-Spirostan-3β,6β-diol 6

Method I. A mixture of the dione **5** (1.5 g, 3.5 mmol), ethanol (150 mL) and PtO_2 (300 mg) was shaken under hydrogen (20 psi) for 4 h. The resulting solution was filtered and the solvent evaporated to afford 1.3 g (86%) of the diol **6**.

Method II. $NaBH_4$ (2.12 g, 56 mmol) was added to a cooled (10 °C), stirred solution of the dione **5** (6 g, 14 mmol) in a mixture of methanol (80 mL) and 1,4-dioxane (30 mL). Stirring was maintained for 30 min, the mixture was poured into cold water, and the solid was filtered off with suction, and dried at 60 °C to afford 4.5 g (74%) of the diol **6**, mp 240.9–242.1 °C (from acetone) (Calc. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25%. Found: C, 74.72; H, 10.33%); IR (ν_{max}/cm^{-1}) 3672 and 3416 (νOH), 2951 and 2930 (νCsp^3-H), 1454 and 1379 (δCH), 1060, 1043 (νC-O), 981, 920, 900 and 875 (spiroketal); ^{1}H NMR (δ) 0.79 (3 H, d, J 5.7, H_3-27), 0.81 (3 H, s, H_3-18), 0.97 (3 H, d, J 6.9, H_3-21), 1.03 (3 H, s, H_3-19), 3.36 (1 H, 26-H_{ax}), 3.48 (1 H, 26-H_{eq}), 3.63 (m, 3-H), 3.77 (m, 6-H), 4.40 (1 H, m, 16-H); ^{13}C NMR (δ) C-1 38.6, C-2 31.2, C-3 71.4, C-4 35.0, C-5 47.6, C-6 71.5, C-7 39.6, C-8 30.1, C-9 54.4, C-10 35.6, C-11 21.0, C-12 40.1, C-13 40.7, C-14 56.2, C-15 31.7, C-16 81.0, C-17 62.2, C-18 16.6, C-19 15.6, C-20 41.7, C-21 14.5, C-22 109.6, C-23 31.4, C-24 28.8, C-25 30.0, C-26 67.0, C-27 17.1.

(25*R*)-3β-Hydroxy-5α-spirostan-6-one 1 (laxogenin)

NBS (500 mg) was added to a solution of the diol **6** (500 mg, 1.16 mmol) in a mixture of acetone (50 mL), water (2.5 mL) and acetic acid (0.4 mL). The mixture was stirred for 45 min, poured into saturated aq. NaCl, and extracted with ethyl acetate (3 × 20 mL). The combined organic solution was washed successively with aq. $NaHCO_3$ (2 × 10 mL) and aq. NaCl (2 × 10 mL), dried with Na_2SO_4, and the solvent was evaporated to afford 456 mg (92%) of laxogenin **1**, mp 209.2–210.8 °C (from acetone–heptane) (lit.,[13] 210–212 °C) (Calc. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.35; H, 9.91%); IR (ν_{max}/cm^{-1}) 3430 (νOH), 2950, 2929 and 2906 (νCsp^3-H), 1706 (νC=O), 1478 and 1379 (δC-H), 1063, 1054 and 1010 (νC-O), 984, 920, 900 and 865 (spiroketal); ^{1}H NMR (δ) 0.77 (3 H, s, H_3-18), 0.77 (3 H, s, H_3-19), 0.79 (3 H, d, J 6.3, H_3-27), 0.97 (3 H, d, J 6.7, H_3-21), 2.20 (1 H, dd, J 3.0 and J 12.8, 5-H), 3.35 (1 H, t, J 10.4, 26-H_{ax}), 3.48 (1 H, m, 26-H_{eq}), 3.56 (1 H, m, 3-H), 4.41 (1 H, m, 16-H); ^{13}C NMR (δ) C-1 36.6, C-2 30.6, C-3 70.5, C-4 30.0, C-5 56.8, C-6 210.6, C-7 46.7, C-8 37.3, C-9 53.9, C-10 40.9, C-11 21.3, C-12 39.5, C-13 40.9, C-14 56.5, C-15 31.5, C-16 80.4, C-17 62.0, C-18 16.4, C-19 13.2, C-20 41.6, C-21 14.4, C-22 109.3, C-23 31.3, C-24 28.7, C-25 30.2, C-26 66.8, C-27 17.1.

(25*R*)-5α-Spirostan-3β,6β-diyl diacetate 7

DMAP (50 mg) and acetic anhydride (20 mL) were added to a solution of the diol **6** (10 g, 23.1 mmol) in dry pyridine (50 g) and the mixture was maintained at 80 °C for 24 h before being poured into ice–water. The solid was filtered off with suction, washed with abundant water, and dried in an oven to afford 11.6 g (97%) of the diacetate **7**, mp 159.1–161.5 °C (from acetone–heptane) (Calc. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.95; H, 9.42%); IR (ν_{max}/cm^{-1}) 2949, 2874 and 2855 (νCsp^3-H), 1456 and 1365 (δCH), 1736 (νC=O), 1238 (νC-O), 1053 and 1028 (νC-O), 981, 920, 899 and 860 (spiroketal); ^{1}H NMR (δ) 0.79 (3 H, d, J 7.7, H_3-27), 0.81 (3 H, s, H_3-18), 0.96 (3 H, d, J 6.8, H_3-21), 1.03 (3 H, s, H_3-19), 2.01 (3 H, s, Me acetyl), 2.05 (3 H, s, Me acetyl), 3.36 (1 H, t, J 7.3, 26-H_{ax}), 3.46 (1 H, dd, 26-H_{eq}), 4.38 (1 H, m, 16-H), 4.71 (1 H, m, 3-H), 4.96 (1 H, m, 6-H); ^{13}C NMR (δ) C-1 37.9, C-2 27.2, C-3 72.9, C-4 30.8, C-5 46.0, C-6 73.2, C-7 36.4, C-8 30.5, C-9 53.7, C-10 35.5, C-11 20.6, C-12 39.7, C-13 40.5, C-14 55.7, C-15 31.6, C-16 80.5, C-17 62.0, C-18 16.4, C-19 15.0, C-20 41.5, C-21 14.4, C-22 109.8, C-23 31.3, C-24 28.7, C-25 30.2, C-26 66.6, C-27 17.0, (Me acetyl) 21.2, (C=O acetyl) 170.3.

(25*R*)-3β,6β-Diacetoxy-5α-spirostan-23-one 8

To a well stirred solution of the diacetate **7** (1 g, 1.9 mmol) in acetic acid (20 mL) were added $NaNO_2$ (1.5 g) and $BF_3{\cdot}Et_2O$ (1.5 mL) in small portions over a period of 1 h. Stirring was maintained for an additional 30 min, when the mixture was poured into cool water and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layer was washed with water (2 × 10 mL), dried over Na_2SO_4, and the solvent was evaporated off in vacuum. The syrupy product was dissolved in benzene (10 mL) and stored for 1 h in a chromatographic column packed with alumina (40 g) (Brockman activity III) before elution with heptane–ethyl acetate (5:1). Evaporation of the solvent afforded 689 mg (68%) of the ketone **8**, mp 202.5–203.8 °C (from ethyl acetate–heptane) (Calc. for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74%. Found: C, 70.05; H, 8.82%); IR (ν_{max}/cm^{-1}) 2962, 2946 and 2917 (νCsp^3-H), 1729 (νC=O), 1458 and 1378 (δCH), 1251, 1238 (νC-O acetate), 1052 and 1027 (νC-O), 966 (spiroketal); ^{1}H NMR (δ) 0.80 (3 H, s, H_3-18), 0.93 (3 H, d, J 7.1, H_3-21), 0.94 (3 H, d, J 6.4, H_3-27), 1.03 (3 H, s, H_3-19), 2.05 (3 H, s, Me acetyl), 2.02 (3 H, s, Me acetyl), 2.44 (2 H, 24-H_2), 2.89 (1 H, q, J 6.8, H-20), 3.57 (1 H, dd, J 4.1 and J 11.1, 26-H_{eq}), 3.79 (1 H, t, J 11.1, 26-H_{ax}), 4.58 (1 H, m, 16-H_{ax}), 4.72 (1 H, m, 3-H_{ax}), 4.94 (1 H, m, 6-H_{eq}); ^{13}C NMR (δ) C-1 37.9, C-2 27.2, C-3 73.3, C-4 30.8, C-5 46.1, C-6 73.0, C-7 36.3, C-8 30.5, C-9 53.7, C-10 35.5, C-11 20.6, C-12 39.5, C-13 41.0, C-14 55.8, C-15 31.6, C-16 83.0, C-17 61.6, C-18 16.2, C-19 15.1, C-20 34.7, C-21 14.3, C-22 109.7, C-23 201.7, C-24 45.1, C-25 35.8, C-26 65.5, C-27 17.0, (Me acetyl) 21.3, (C=O acetyl) 170.4.

(25*R*)-3β,6β-Dihydroxy-5α-spirostan-23-one 9

A solution of the diacetylated ketone **8** (400 mg, 0.754 mmol) and KOH (400 mg) in methanol (40 mL) was refluxed for 2 h. Ethyl acetate (80 mL) was added and the solution was washed with saturated aq. NaCl (5 × 10 mL), dried with Na_2SO_4, and the solvent was evaporated off to afford 299 mg (89%) of the dihydroxy ketone **9**, mp 241–243 °C (from acetone–heptane) (Calc. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.26; H, 9.89%); IR (ν_{max}/cm^{-1}) 3550 and 3397 (νOH), 2970, 2952 and 2935 (νCsp^3-H), 1731 (νC=O), 1457 and 1361 (δCH), 1064, 1047 and 1025 (νC-O), 962 (spiroketal).

(25*R*)-3β-Hydroxy-5α-spirostan-6,23-dione 10

NBS (300 mg, 1.68 mmol) was added to a solution of the dihydroxylated ketone **9** (300 mg, 0.672 mmol) in a mixture of acetone (45 mL), water (2 mL) and acetic acid (0.3 mL). The mixture was stirred for 45 min, poured into saturated aq. NaCl, and extracted with ethyl acetate (3 × 20 mL). The combined organic solution was washed successively with aq. $NaHCO_3$ (2 × 10 mL) and aq. NaCl (2 × 10 mL), dried with Na_2SO_4, and the solvent was evaporated off to afford 274 mg (92%) of the hydroxylated dione **10**, mp 234.2–235.9 °C (from acetone–heptane) (Calc. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 72.88; H, 9.16%); IR (ν_{max}/cm^{-1}) 3525 (νOH), 2937, 2917 and 2883 (νCsp^3-H), 1727 (νC^{23}=O), 1706 (νC^6=O), 1454 and 1378

(δCH), 1061 and 1030 (νC-O), 995 and 968 (spiroketal); ^{1}H NMR (δ) 0.77 (3 H, s, H_3-19), 0.78 (3 H, s, H_3-18), 0.94 (3 H, d, J 6.8, H_3-21), 0.94 (3 H, d, J 6.8, H_3-27), 2.20 (1 H, dd, J 2.7 and J 12.4, 5-H), 2.46 (2 H, m, 24-H), 2.90 (1 H, q, J 6.7, H-20), 3.59 (1 H, dd, J 4.4 and J 11.4, 26-H_{eq}), 3.63 (1 H, m, 3-H), 3.78 (1 H, t, J 11.1, 26-H_{ax}), 4.63 (1 H, m, 16-H); ^{13}C NMR (δ) C-1 36.6, C-2 30.7, C-3 70.6, C-4 30.0, C-5 56.8, C-6 210.3, C-7 46.7, C-8 37.3, C-9 53.9, C-10 40.9, C-11 21.3, C-12 39.2, C-13 41.4, C-14 56.6, C-15 31.5, C-16 82.9, C-17 61.7, C-18 16.1, C-19 13.2, C-20 34.7, C-21 14.4, C-22 109.8, C-23 201.7, C-24 45.2, C-25 35.8, C-26 65.7, C-27 17.0.

(23*S*,25*R*)-5α-Spirostan-3β,6β,23-triol 3,6-diacetate 11

$NaBH_4$ (180 mg, 4.76 mmol) was slowly added to a cool (10 °C) solution of the diacetylated ketone **8** (700 mg, 1.32 mmol) in methanol (40 mL). The mixture was stirred for 30 min, water (2 mL) was added, and stirring was maintained for an additional 10 min, followed by neutralisation with acetic acid and evaporation of the solvent. The residue was dissolved in ethyl acetate and the solution was washed with aq. NaCl, dried with Na_2SO_4, and evaporated to afford 620 mg (88%) of the diacetylated triol **11**, mp 220.9–223.8 °C (from acetone–heptane) (Calc. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 69.83; H, 9.14%); IR (ν_{max}/cm^{-1}) 3500 and 3400 (νOH), 2954 and 2931 (νCsp^3-H), 1736 (νC=O), 1456 and 1380 (δCH), 1250 and 1240 (νC-O acetate), 1062, 1054 and 1029 (νC-O), 966 (spiroketal); ^{1}H NMR (δ) 0.82 (3 H, d, J 6.6, H_3-27), 0.85 (3 H, s, H_3-18), 0.95 (3 H, d, J 7.0, H_3-21), 1.03 (3 H, s, H_3-19), 2.02 (3 H, s, Me acetyl), 2.05 (3 H, s, Me acetyl), 2.54 (1 H, m, H-20), 3.25 (1 H, t, J 11.1, 26-H_{ax}), 3.41 (1 H, dd, 26-H_{eq}), 3.48 (1 H, 23-H_{ax}), 4.45 (1 H, m, 16-H_{ax}), 4.71 (1 H, m, 3-H_{ax}), 4.95 (1 H, m, 6-H_{eq}); ^{13}C NMR (δ) C-1 38.0, C-2 27.3, C-3 73.3, C-4 30.9, C-5 46.2, C-6 73.1, C-7 36.5, C-8 30.4, C-9 53.8, C-10 35.6, C-11 20.7, C-12 39.9, C-13 41.0, C-14 55.7, C-15 31.7, C-16 81.4, C-17 61.5, C-18 16.6, C-19 15.1, C-20 35.6, C-21 14.5, C-22 110.5, C-23 67.0, C-24 38.5, C-25 30.8, C-26 65.9, C-27 16.7, (Me acetyl) 21.3, (C=O acetyl) 170.5.

(23*S*,25*R*)-5α-Spirostan-3β,6β,23-triol 12

A solution of the diacetylated triol **11** (400 mg, 0.752 mmol) and KOH (400 mg) in methanol (20 mL) was refluxed for 2 h. Ethyl acetate (80 mL) was added and the solution was washed with saturated aq. NaCl (5 × 10 mL), dried with Na_2SO_4, and the solvent was evaporated off to afford 304 mg (90%) of the triol **12**, mp 247.8–250.1 °C (from acetone–heptane) (Calc. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.29; H, 9.91%); IR (ν_{max}/cm^{-1}) 3418 (νOH), 2947 and 2932 (νCsp^3-H), 1064 and 1043 (νC-O), 962 (spiroketal).

(23*S*,25*R*)-3β,23-Dihydroxy-5α-spirostan-6-one 13

NBS (300 mg, 1.68 mmol) was added to a solution of the triol **12** (300 mg, 0.670 mmol) in a mixture of acetone (45 mL), water (2 mL), and acetic acid (0.3 mL). The mixture was stirred for 45 min, poured into saturated aq. NaCl, and extracted with ethyl acetate (3 × 20 mL). The organic solution was washed successively with aq. $NaHCO_3$ (2 × 10 mL) and aq. NaCl (2 × 10 mL), dried with Na_2SO_4, and the solvent was evaporated off to afford 267 mg (89%) of the dihydroxylated ketone **13**, mp 251.9–253.6 °C (from acetone–heptane) (Calc. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.22; H, 9.90%); IR (ν_{max}/cm^{-1}) 3456 and 3401 (νOH), 2954 and 2927 (νCsp^3-H), 1702 (νC=O), 1057 (δCH), 1099, 1074 and 1060 (νC-O), 993 and 964 (spiroketal); ^{1}H NMR (δ) 0.77 (3 H, s, H_3-19), 0.82 (3 H, s, H_3-18), 0.82 (3 H, d, J 6.3, H_3-27), 0.96 (3 H, d, J 7.0, H_3-21), 2.20 (1 H, dd, 5-H_{ax}), 2.56 (1 H, m, H-20), 3.24 (1 H, t, J 10.9, 26-H_{ax}), 3.40 (1 H, dd, J 2.9 and J 10.5, 26-H_{eq}), 3.50–3.56 (2 H, m, 3-H_{ax}, 3-H_{ax}), 4.47 (1 H, m, 16-H); ^{13}C NMR (δ) C-1 36.6, C-2 30.6, C-3 70.5, C-4 29.9, C-5 56.8, C-6 210.5, C-7 46.7, C-8 37.2, C-9 53.8, C-10 40.9, C-11 21.3, C-12 39.5, C-13 41.4, C-14 56.4, C-15 31.5, C-16 81.1, C-17 61.5, C-18 16.4, C-19 13.2, C-20 35.5, C-21 14.0, C-22 110.6, C-23 67.0, C-24 38.4, C-25 30.8, C-26 65.9, C-27 16.5.

(23*S*,25*R*)-3β,23-Diacetoxy-5α-spirostan-6-one 14

DMAP (10 mg) was added to a solution of the dihydroxylated ketone **13** (300 mg, 0.673 mmol) in a mixture of pyridine (5 mL) and acetic anhydride (1 mL). The mixture was stirred overnight, poured into crushed ice, and extracted with ethyl acetate (5 × 20 mL). The organic solvent was washed successively with 10% aq. $CuSO_4$ (5 × 15 mL) and water (3 × 15 mL), dried with Na_2SO_4, evaporated to afford 342 mg (96%) of the diacetylated ketone **14**, mp 187.5–189.7 °C (from acetone–heptane) (Calc. for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74%. Found: C, 70.10; H, 8.80%); IR (ν_{max}/cm^{-1}) 2950 and 2939 (νCsp^3-H), 1739 and 1727 (νC=O acetyl), 1716 (νC^6=O), 1454 and 1371 (δCH), 1238 (νC-O), 1058, 1035 and 1024 (νC-O), 966 (spiroketal); ^{1}H NMR (δ) 0.79 (3 H, s, H_3-19), 0.82 (3 H, s, H_3-18), 0.83 (3 H, d, J 6.0, H_3-27), 0.96 (3 H, d, J 7.0, H_3-21), 2.03 (3 H, s, Me acetyl), 2.02 (3 H, s, Me acetyl), 3.34 (1 H, t, J 10.9, 26-H_{ax}), 3.44 (1 H, dd, J 6.5 and J 11.1, 26-H_{eq}), 4.46 (1 H, m, 16-H), 4.66 (1 H, m, 3-H), 4.82 (1 H, dd, J 4.7 and J 11.8, 23-H_{ax}); ^{13}C NMR (δ) C-1 36.1, C-2 26.6, C-3 72.5, C-4 25.9, C-5 56.2, C-6 209.5, C-7 46.4, C-8 37.2, C-9 53.5, C-10 40.6, C-11 21.0, C-12 39.1, C-13 41.2, C-14 56.2, C-15 31.3, C-16 80.6, C-17 61.3, C-18 16.2, C-19 12.9, C-20 35.8, C-21 13.9, C-22 108.4, C-23 68.4, C-24 33.8, C-25 30.5, C-26 65.5, C-27 15.8, (Me acetyl) 21.4, 170.5 (C=O acetyl).

(23*S*,25*R*)-23-Acetoxy-3β-hydroxy-5α-spirostan-6-one 15

The diacetylated ketone **14** (300 mg, 0.566 mmol) was added to a saturated solution of Na_2CO_3 in methanol (10 mL), the mixture was stirred at 0 °C for 3 h, and then poured into saturated aq. NaCl and extracted with ethyl acetate (4 × 15 mL). The extract was dried with Na_2SO_4 and evaporated to a syrup, which consisted of a mixture of the starting material and the desired monoacetate. Chromatographic separation on silica gel (heptane–ethyl acetate 95 : 5 to 70 : 30) afforded 107 mg (39%) of the monoacetate **15**, mp 195–196 °C (from acetone–heptane) (Calc. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found: C, 71.22; H, 9.16%); IR (ν_{max}/cm^{-1}) 3424 (νOH), 2954 and 2931 (νCsp^3-H), 1745 (νC=O acetyl), 1700 (νC^6=O), 1454 and 1376 (δCH), 1253 (νC-O), 1060 and 1024 (νC-O), 962 (spiroketal); ^{1}H NMR (δ) 0.77 (s, H_3-19), 0.82 (s, H_3-18), 0.83 (d, J 6.9, H_3-27), 0.96 (d, J 7.0, H_3-21), 2.03 (s, Me acetyl), 3.34 (1 H, t, J 10.9, 26-H_{ax}), 3.42 (1 H, dd, J 4.8 and J 11.8, 26-H_{eq}), 3.55 (1 H, m, 3-H), 4.47 (1 H, m, 16-H), 4.82 (1 H, dd, J 4.8 and J 11.8, 23-H_{ax}); ^{13}C NMR (δ) C-1 36.6, C-2 30.7, C-3 70.5, C-4 30.0, C-5 56.8, C-6 210.5, C-7 46.7, C-8 37.4, C-9 53.8, C-10 40.9, C-11 21.3, C-12 39.4, C-13 41.4, C-14 56.5, C-15 31.2, C-16 81.1, C-17 61.5, C-18 16.4, C-19 13.2, C-20 36.0, C-21 14.1, C-22 108.6, C-23 68.6, C-24 34.0, C-25 30.7, C-26 65.8, C-27 16.1, (Me acetyl) 21.2, (C=O acetyl) 170.6.

Acknowledgements

Thanks are due to Mrs Rosa Arteaga for correction of the manuscript.

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