Synthesis of 6-Deoxohomodolichosterone, a New Plant-growth-promoting Steroid

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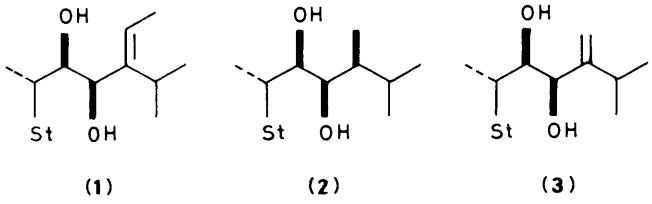
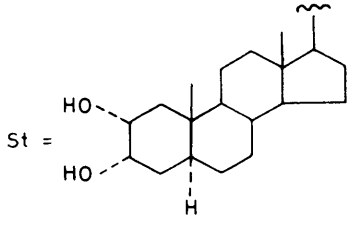
6-Deoxohomodolichosterone (**1**), a new member of the brassinosteroid family, was synthesized in twelve steps from stigmasterol (**4**). Birch reduction of the dienone (**5**) gave directly 5α-stigmast-22-en-3β-ol (**6**), the mesylate of which was treated with lithium bromide in refluxing dimethylformamide. Selective oxidation of the 2-ene function of the resulting 2,22-diene (**7**), followed by acetonide formation, provided the acetonide 22-alkene (**8**). Epoxide-ring opening of the (22*R*,23*R*)-epoxide (**9**), derived from (**8**), with phenylselenolate anion, treatment with 30% hydrogen peroxide, and epoxidation with peracid afforded the 22-hydroxy-23,24-epoxide (**15**). Ready cleavage of the epoxide ring of compound (**15**) with aluminium isopropoxide and deprotection yielded 6-deoxohomodolichosterone (**1**).

6-Deoxohomodolichosterone (**1**) was isolated from the immature seeds of *Phaseolus vulgaris* cv. Kentucky Wonder as a new steroidal plant-growth promoter.[1] The gross structure of compound (**1**) was proposed by the electron-impact mass spectrum (e.i.m.s.) of its bismethaneboranate derivative, but its stereochemistry remains to be clarified. In order to determine the structure of the new sterol, our efforts have been directed to its synthesis. Since 6-deoxocastasterone (**2**) and 6-deoxodolichosterone (**3**) were also isolated from the same seeds,[1,2] we have synthesized one possible compound, (22*R*,23*R*)-5α-stigmast-24(28)(*E*)-ene-2α,3α,22,23-tetraol (**1**). G.c.–e.i.m.s. comparison of the synthetic and natural 6-deoxohomodolichosterone (**1**) confirmed that the structure of the natural compound is the one shown in structure (**1**).† In this paper we report the details of our synthesis of 6-deoxohomodolichosterone (**1**), in which the requisite functional groups for this compound were introduced first into the steroidal nucleus and then into the side-chain of the starting material, stigmasterol (**4**).

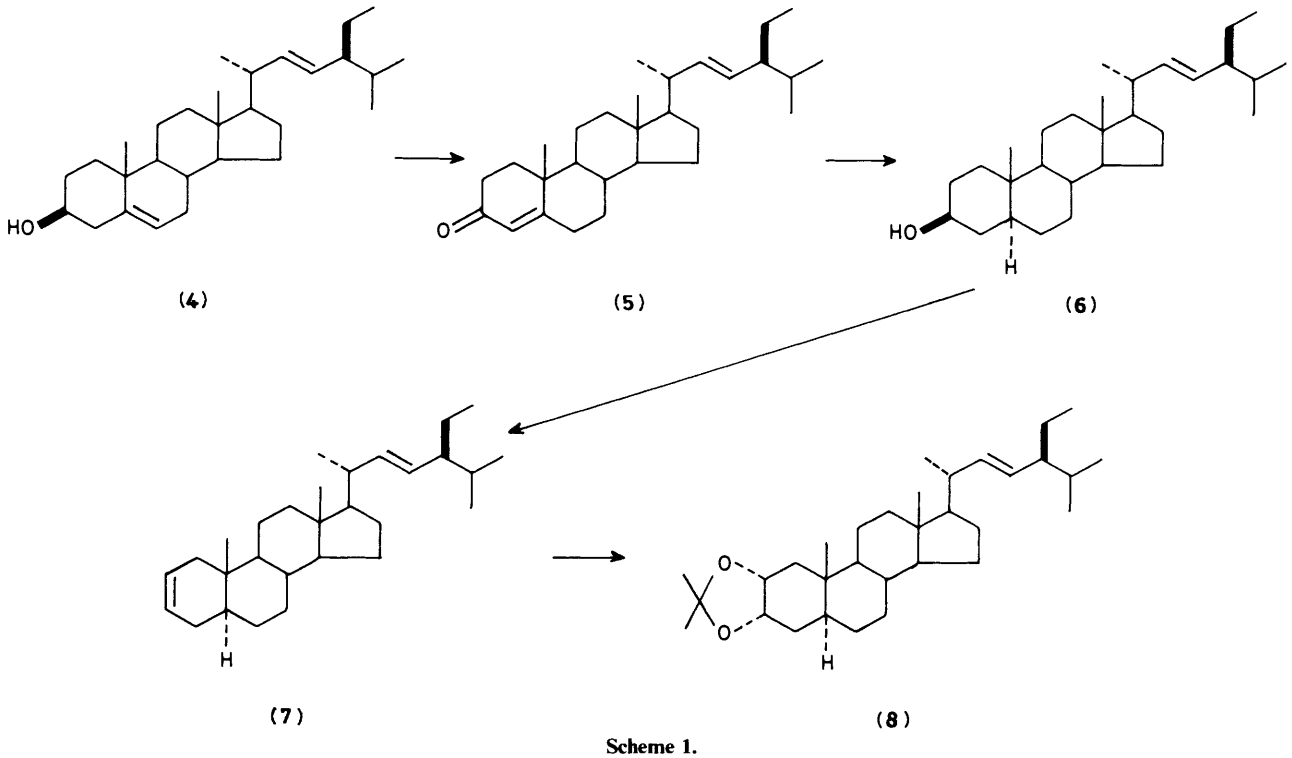




Transformation of the A/B ring part of stigmasterol (**4**) into that of 6-deoxohomodolichosterone (**1**) was first carried out, as follows. Oppenauer oxidation of (**4**) gave the enone (**5**), which was then directly reduced with lithium and ammonia in dioxane–methanol at −78 °C to provide (24*S*)-5α-stigmast-22-(*E*)-en-3β-ol (**6**) in 64% yield from stigmasterol (**4**). This was submitted to methanesulphonation and then treatment with lithium bromide in refluxing dimethylformamide (DMF).[3] The resulting 2,22-diene (**7**) contaminated with a small amount of the corresponding 3,22-diene was regio- and stereo-selectively hydroxylated with a catalytic amount of osmium tetraoxide and *N*-methylmorpholine *N*-oxide in aqueous tetrahydrofuran (THF).[3] Subsequent acetonide formation afforded the 2α,3α-diol acetonide (**8**) in 58% yield from (**6**) (Scheme 1). The regioisomeric 3α,4α-diol acetonide was removed by column chromatography.

Having constructed the A/B ring part of 6-deoxohomodolichosterone (**1**), we focussed our attention on introduction of the side-chain functions of the target molecule (**1**) into the 22-ene compound (**8**). Epoxidation of compound (**8**) with *m*-chloroperbenzoic acid (MCPBA) gave two separable epoxides, the less polar (22*S*,23*S*)-epoxide (**10**) [40%; δ($CDCl_3$) 2.45 (2 H, m, 22- and 23-H)] and the more polar (22*R*,23*R*)-epoxide (**9**) [47%; δ($CDCl_3$) 2.45 (1 H, m, 22- or 23-H), 2.73 (1 H, dd, *J* 7 and 2 Hz, 23- or 22-H)]. The stereochemical assignment of these two epoxides was based on comparison with ^1H n.m.r. data of the epoxidic protons of our previously reported (22*S*,23*S*)- and (22*R*,23*R*)-3β-acetoxy-22,23-epoxy-5α-stigmastan-6-one.[4] The (22*R*,23*R*)-epoxide ring of compound (**9**) was cleaved by phenylselenolate anion[5] in refluxing butan-1-ol to give a mixture of the selenides (**11**) and (**12**), which without isolation was further treated with 30% H_2O_2 at room temperature. The resulting products contained the allylic alcohols (**13**) and (**14**) and the hydroxy epoxides (**15**) and (**16**). Further treatment of the mixture with MCPBA converted alkenes (**13**) and (**14**) into epoxides (**15**) and (**16**), respectively. At this stage, separation by column chromatography was carried out. The more polar hydroxy-epoxide (**15**) [32%; δ($CDCl_3$) 2.85 (1 H, d, *J* 6 Hz, 23-H)] and the less polar regioisomer (**16**) [9%; δ($CDCl_3$) 3.10 (1 H, d, *J* 8 Hz, 22-H)] were obtained along with recovery of the 22,23-epoxide (**9**) [55%]. Comparison of the ^1H n.m.r. data of the hydroxy epoxides with those of the reference compounds[6,7] assigned structures (**15**) and (**16**) to the 23,24- and 20,22-epoxide, respectively. The 22-hydroxy-23,24-epoxide (**15**) was treated with aluminium isopropoxide[8] in refluxing toluene and then deprotected with aqueous acetic acid at 60 °C to provide a 77:23 mixture of 6-deoxohomodolichosterone (**1**) and its 24(28)(*Z*)-isomer (**18**) (Scheme 2); the compounds were determined by g.l.c. as bismethaneboronate derivatives[9] and ^1H

† Identification of natural 6-deoxohomodolichosterone (**1**) will be reported elsewhere.



(4) (5) (6) (7) (8)

Scheme 1.

n.m.r. (400 MHz) spectroscopy. Recrystallization from methanol gave fine needles of 6-deoxohomodolichosterone (**1**) [33% from (**15**), m.p. 225—227 °C]. The 22*R*,23*R*,24(28)(*E*)-configuration of the side-chain and the 2α,3α-diol and 5α-ring junction of the A/B ring part of the synthetic 6-deoxohomodolichosterone (**1**) were confirmed by comparison of its ^{1}H n.m.r. data (see Experimental section) with those of homodolichosterone[6] and 6-deoxocastasterone,[7] respectively.

Experimental

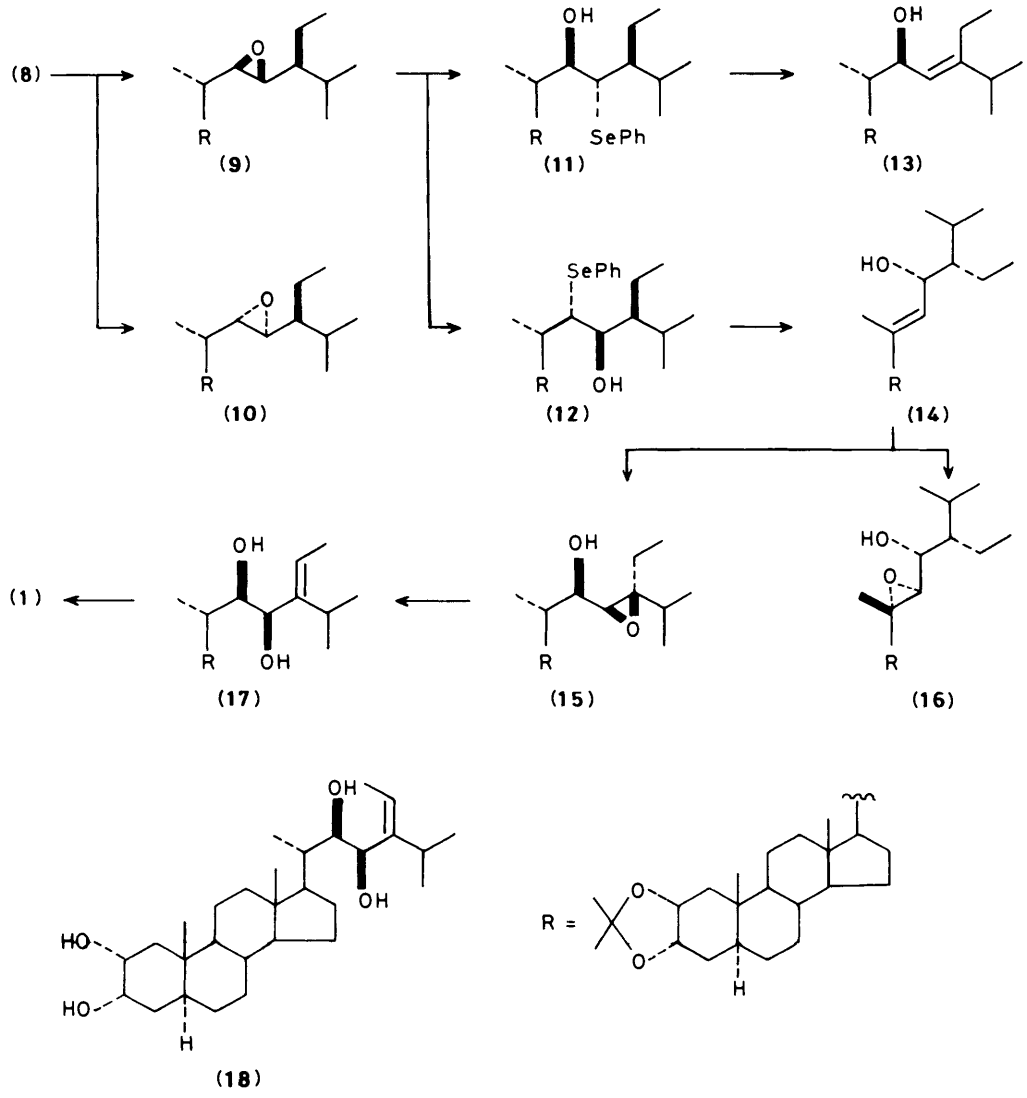
^{1}H N.m.r. spectra were recorded with a Hitachi R-24A (60 MHz) spectrometer for samples in $CDCl_3$ solution with tetramethylsilane as an internal standard unless otherwise noted. Mass spectra were taken with Shimadzu LKB 9000S and GC-MS 9020 mass spectrometers at 20 eV. Kieselgel $60F_{254}$ (Merck) was used for analytical t.l.c. Column chromatography was carried out with Kieselgel 60 (70—230 mesh, Merck). Work-up refers to dilution with water, extraction with an organic solvent indicated in parenthesis, washing of the extract to neutrality, drying ($MgSO_4$), and removal of the solvent under reduced pressure.

(24S)-*Stigmasta*-4,22(E)-*dien*-3-*one* (**5**).—A solution of stigmasterol (**4**) (13.5 g, 32.77 mmol) in toluene (300 ml) and 1-methyl-4-piperidone (30 ml) was refluxed under a Dean–Stark trap until *ca.* 30 ml of distillate had collected. To the remaining solution was added aluminium isopropoxide (10.2 g, 50 mmol). The mixture was refluxed for 6 h. Work-up (EtOAc) gave a crude product, which was applied to a column of silica gel (4.5 cm i.d. × 26 cm). Elution with benzene–EtOAc (50:1) gave the *dienone* (**5**) (11.7 g, 87%), m.p. 119—122 °C (from acetone); δ($CDCl_3$) 0.74 (3 H, s, 18-H_3), 1.07 (3 H, s, 19-H_3), 5.30 (2 H, m, 22- and 23-H), and 5.70 (1 H, br s, 4-H) (Found: C, 84.6; H, 11.5. $C_{29}H_{46}O$ requires C, 84.81; H, 11.29%).

(24S)-5α-*Stigmast*-22(E)-*en*-3β-*ol* (**6**).—To a solution of lithium (2.0 g) in liquid ammonia (350 ml) was added a solution of the dienone (**5**) (4.75 g, 11.59 mmol) in dioxane (220 ml) at −78 °C under argon. The mixture was stirred at −78 °C for 1 h. To the reaction mixture was added methanol until all the lithium had reacted, and then a new portion of lithium (2.0 g) was added to maintain the blue colour for 1 h. The excess of lithium was destroyed with dry solid ammonium chloride. The cooling bath was removed and the ammonia was allowed to evaporate off. The crude product obtained by extraction with diethyl ether was chromatographed on silica gel (4.5 cm i.d. × 30 cm) with benzene–EtOAc (10:1) to give the 3β-*sterol* (**6**) (3.5 g, 73%), m.p. 159—161 °C (from acetone); δ($CDCl_3$) 0.65 (3 H, s, 18-H_3), 0.79 (3 H, s, 19-H_3), 3.50 (1 H, m, 3-H), and 5.29 (2 H, m, 22- and 23-H); e.i.m.s. *m/z* 414 (M^+) (Found: C, 83.8; H, 11.9. $C_{29}H_{50}O$ requires C, 83.99; H, 12.15%).

(24S)-5α-*Stigmasta*-2,22(E)-*diene* (**7**).—A solution of the 3β-sterol (**6**) (3.1 g, 7.49 mmol) in pyridine (60 ml) was treated with methanesulphonyl chloride (3 ml) at room temperature for 1 h. Work-up (EtOAc) gave the corresponding mesyl derivative, which in DMF (50 ml) was treated with lithium bromide (2.3 g, 26.44 mmol) under reflux for 1 h. Work-up (EtOAc) gave a crude product, which was applied to a column of silica gel (2.5 cm i.d. × 20 cm). Elution with hexane–benzene (1:1) gave the *diene* (**7**) (2.4 g, 81%), m.p. 98—100 °C (from acetone); δ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 0.78 (3 H, s, 19-H_3), 5.30 (2 H, m, 22- and 23-H), and 5.62 (2 H, m, 2- and 3-H) (Found: C, 87.85; H, 12.0. $C_{29}H_{48}$ requires C, 87.80; H, 12.20%). This was contaminated with a small amount of the corresponding 3,22-diene.

(24S)-2α,3α-*Isopropylidenedioxy*-5α-*stigmast*-22(E)-*ene* (**8**).—A solution of the diene (**7**) (2.3 g, 5.81 mmol) in aqueous THF (20 ml) was treated with osmium tetraoxide (20 mg) and *N*-methylmorpholine *N*-oxide (2.6 g, 19.26 mmol) at room temperature for 5 h. Work-up (CH_2Cl_2) gave a crude product, which in acetone (50 ml) was treated with toluene-*p*-sulphonic acid (50 mg) at room temperature for 14 h. Work-up (diethyl ether) and chromatography on silica gel (2.5 cm i.d. × 27 cm) with benzene gave the *acetonide* (**8**) (1.95 g, 71%), amorphous,



Scheme 2.

δ($CDCl_3$) 0.68 (3 H, s, 18-H_3), 0.72 (3 H, s, 19-H_3), 1.33 and 1.50 (6 H, 2 × s, acetonide), 3.80—4.30 (2 H, m, 2- and 3-H), and 5.30 (2 H, m, 22- and 23-H); e.i.m.s. *m/z* 470 (M^+) (Found: C, 81.5; H, 11.7. $C_{32}H_{54}O_2$ requires C, 81.64; H, 11.56%). The more polar, regioisomeric 3α,4α-diol acetonide was removed by column chromatography.

(22R,23R)- *and* (22S,23S)-22,23-*Epoxy*-2α,3α-*isopropylidenedioxy*-5α-*stigmastane* (**9**) *and* (**10**).—A solution of the acetonide (**8**) (1.94 g, 4.13 mmol) in chloroform (15 ml) was treated with MCPBA (860 mg, 4.97 mmol) at room temperature for 20 h. Calcium hydroxide (2.0 g) was added to the reaction mixture, which was then stirred at room temperature for 1 h. Filtration, and removal of the solvent under reduced pressure, gave crude products, which were applied to a column of silica gel (2.5 cm i.d. × 37 cm). Elution with hexane–EtOAc (20:1) gave the less polar (22*S*,23*S*)-epoxide (**10**) (812 mg, 40%), amorphous, δ($CDCl_3$) 0.65 (3 H, s, 18-H_3), 1.00 (3 H, s, 19-H_3), 1.32 and 1.49 (6 H, 2 × s, acetonide), 2.45 (2 H, m, 22- and 23-H), and 3.90—4.40 (2 H, m, 2- and 3-H); e.i.m.s. *m/z* 486 (M^+).

Further elution with the same solvent gave the *more polar* (22R,23R)-*epoxide* (**9**) (958 mg, 47%), amorphous, δ($CDCl_3$) 0.65 (3 H, s, 18-H_3), 0.98 (3 H, s, 19-H_3), 1.31 and 1.49 (6 H, 2 × s, acetonide), 2.45 (1 H, m, 22- or 23-H), 2.73 (1 H, dd, *J* 7 and 2 Hz, 23- or 22-H), and 3.90—4.40 (2 H, m, 2- and 3-H); e.i.m.s. *m/z* 486 (M^+) (Found: C, 79.05; H, 11.3. $C_{32}H_{54}O_3$ requires C, 78.96; H, 11.18%).

(20S,22S,23R,24S)-20,22-*Epoxy*-2α,3α-*isopropylidenedioxy*-5α-*stigmastan*-23-*ol* (**16**) *and* (22R,23S,24S)-23,24-*Epoxy*-2α,3α-*isopropylidenedioxy*-5α-*stigmastan*-22-*ol* (**15**).—To a suspension of diphenyl diselenide (1 248 mg, 4.0 mmol) in butan-1-ol (20 ml) was added sodium borohydride (308 mg, 8.08 mmol). The mixture was stirred at room temperature for 20 min, during which time it became colourless. Then, a solution of the epoxide (**9**) (480 mg, 0.988 mmol) in butan-1-ol (10 ml) was added to the reagent solution. The mixture was refluxed for 65 h under argon. Additional phenylselenolate anion solution (8.0 mmol) was then added to the reaction mixture, which was refluxed for a further 40 h. After cooling to 0 °C, the mixture was diluted with diethyl ether. The ethereal phase was washed with water and dried ($MgSO_4$). Removal of the solvent gave a mixture containing the α-hydroxy selenides (**11**) and (**12**). The mixture was dissolved in THF (30 ml)–ethanol (30 ml) and the solution was treated with 30% H_2O_2 (10 ml) at room temperature for 1.5 h. Work-up (diethyl ether) gave products containing the allylic alcohol (**13**), the hydroxy epoxide (**15**), and their regioisomers (**14**) and (**16**). This mixture was further treated with MCPBA

(200 mg) and chloroform (10 ml) at room temperature for 1.5 h. Calcium hydroxide (1.0 g) was added to the reaction mixture, which was then stirred at room temperature for 30 min. Filtration and removal of the solvent gave crude products, which were applied to a column of silica gel (2.5 cm i.d. × 28 cm). Elution with hexane–EtOAc (20:1) gave the starting material (**9**) (264 mg, 55% recovery). Further elution with hexane–EtOAc (10:1) gave the less polar hydroxy epoxide (**16**) (45 mg, 9%), amorphous, δ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 0.72 (3 H, s, 19-H_3), 1.35 (6 H, s, 21-H_3 and acetonide), 1.50 (3 H, s, acetonide), 3.10 (1 H, d, *J* 8 Hz, 22-H), 3.68 (1 H, m, 23-H), and 3.90—4.40 (2 H, m, 2- and 3-H); e.i.m.s. *m/z* 502 (M^+).

Further elution with the same solvent gave the *more polar hydroxy epoxide* (**15**) (158 mg, 32%), m.p. 184—186 °C (from hexane); δ($CDCl_3$) 0.67 (3 H, s, 18-H_3), 0.72 (3 H, s, 19-H_3), 1.33 and 1.50 (6 H, 2 × s), acetonide), 2.85 (1 H, d, *J* 6 Hz, 23-H), 3.58 (1 H, m, 22-H), and 3.90—4.40 (2 H, m, 2- and 3-H); e.i.m.s. *m/z* 502 (M^+) (Found: C, 76.4; H, 10.85. $C_{32}H_{54}O_4$ requires C, 76.45; H, 10.83%).

(22R,23R)-2α,3α-*Isopropylidenedioxy*-5α-*stigmast*-24(28)(E)-*ene*-22,23-*diol* (**17**).—A solution of the hydroxy epoxide (**15**) (80 mg, 0.159 mmol) in toluene (10 ml) was treated with aluminium isopropoxide (42 mg, 0.206 mmol) under reflux for 2 h. Work-up (CH_2Cl_2) gave the diol (**17**) (76 mg), amorphous δ($CDCl_3$) 0.60 (3 H, s, 18-H_3), 0.68 (3 H, s, 19-H_3), 1.31 and 1.49 (6 H, 2 × s, acetonide), 1.68 (3 H, d, *J* 7 Hz, 29-H_3), 2.73 (1 H, sept, *J* 7 Hz, 25-H), 3.65 (1 H, d, *J* 8.5 Hz, 22-H), 3.90 (1 H, d, *J* 8.5 Hz, 23-H), 4.32 (2 H, m, 2- and 3-H), and 5.50 (1 H, q, *J* 7 Hz, 28-H).

(22R,23R)-5α-*Stigmast*-24(28)(E)- *and* -24(28)(Z)-*ene*-2α,3α,22,23-*tetraol* (**1**) *and* (**18**).—The diol acetonide (**17**) (76 mg) was treated with 70% aqueous acetic acid (5 ml) at 60 °C for 1 h. The ice-cooled reaction mixture was neutralized with 5% aqueous NaOH. Work-up (CH_2Cl_2) and chromatography on silica gel (1.5 cm i.d. × 20 cm) with hexane–EtOAc (1:2) gave a 77:23 mixture of 6-deoxohomodolichosterone (**1**) and its 24(28)(*Z*)-isomer (**18**), which was determined by 400 MHz ^{1}H n.m.r. spectroscopy and g.l.c. The g.l.c. retention times [Shimadzu GC-7A; 1% OV-17 (3 mm i.d. × 1 m); column temp. 270 °C; N_2 flow rate 50 ml min^{-1}] of the bismethaneboronate derivatives[9] of compounds (**1**) and (**18**) were 12.3 and 10.0 min (cholesterol, 4.0 min) respectively. Recrystallization from methanol gave 6-*deoxohomodolichosterone* (**1**) [24 mg, 33% from (**15**)], m.p. 225—227 °C (from MeOH); δ($CDCl_3$; 400 MHz) 0.61 (3 H, s, 18-H_3), 0.79 (3 H, s, 19-H_3), 0.92 (3 H, d, *J* 6.5 Hz, 21-H_3), 1.06 (3 H, d, *J* 7.1 Hz, 26-H_3), 1.14 (3 H, d, *J* 7.1 Hz, 27-H_3), 1.71 (3 H, d, *J* 7.1 Hz, 29-H_3), 2.76 (1 H, sept, *J* 7.1 Hz, 25-H), 3.69 (1 H, ddd, *J* 8.5, 3.5, and 0.8 Hz, 22-H), 3.76 (1 H, m, 2β-H), 3.95 (1 H, dd, *J* 8.5 and 4.0 Hz, 23-H), 3.96 (1 H, br s, 3β-H), and 5.51 (1 H, q, *J* 7.1 Hz, 28-H); e.i.m.s. (as bismethaneboronate)[9] *m/z* 510 (M^+, 28%), 467 (76), 413 (6), 365 (2), 356 (18), 343 (20), 313 (74), 283 (12), 273 (14), 255 (4), 235 (6), 222 (28), 167 (78), 138 (100), 96 (38), and 82 (16) (Found: C, 75.2; H, 10.9. $C_{29}H_{50}O_4$ requires C, 75.28; H, 10.89%).

The mother liquor was concentrated to give the enriched 24(28)(*Z*)-isomer (**18**), which was still contaminated with its isomer (**1**). The (*Z*)-isomer (**18**) had the following spectral data: δ($CDCl_3$; 400 MHz) 0.60 (3 H, s, 18-H_3), 0.79 (3 H, s, 19-H_3), 0.93 (3 H, d, *J* 6.5 Hz, 21-H_3), 1.04 (3 H, d, *J* 7 Hz, 26-H_3), 1.07 (3 H, d, *J* 7 Hz, 27-H_3), 1.70 (3 H, d, *J* 7 Hz, 29-H_3), 2.33 (1 H, sept, *J* 7 Hz, 25-H), 3.72—3.80 (2 H, m, 2β- and 22-H), 3.96 (1 H, br s, 3β-H), 4.49 (1 H, d, *J* 9 Hz, 23-H), and 5.54 (1 H, q, *J* 7 Hz, 28-H); e.i.m.s. (as bismethaneboronate)[9] *m/z* 510 (M^+, 24%), 467 (100), 413 (9), 365 (6), 356 (18), 343 (18), 313 (78), 283 (14), 273 (18), 255 (8), 235 (6), 222 (27), 167 (86), 155 (23), 153 (44), 138 (74), 125 (56), 96 (30), and 82 (18).

Acknowledgements

We thank Professor N. Takahashi and Dr. T. Yokota of The University of Tokyo for g.c.–e.i.m.s. comparison of synthetic and natural 6-deoxohomodolichosterone (**1**).

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Received 18*th February* 1986; *Paper* 6/346