Synthesis of (2*R*,3*S*,22*S*,23*S*)-2,3,22,23-Tetrahydroxy-B-homo-7-aza-5α-stigmastan-6-one, an Aza-analogue of Homobrassinolide

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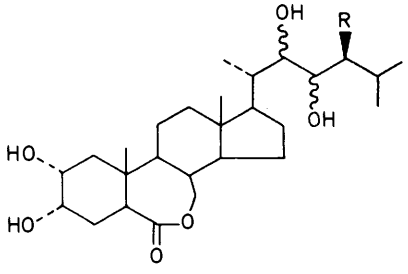
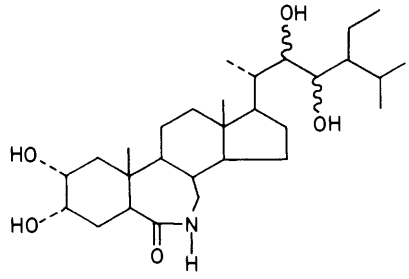
(2*R*,3*S*,22*S*,23*S*)-2,3,22,23-Tetrahydroxy-B-homo-7-aza-5α-stigmastan-6-one, an aza-analogue of homobrassinolide, has been synthesized starting with 5α-stigmasta-2,22-dien-6-one. Osmylation of the trimethylsilyl enol ether of this dienone afforded (2*R*,3*S*,7*S*,22*S*,23*S*)-2,3,7,22,23-pentahydroxy-5α-stigmastan-6-one, which by oxidation of the acyloin group, after protection of the glycolic systems, afforded (by esterification) an aldehydo ester, which is the key intermediate for the production of the final compound by reductive amination.

Since the discovery of brassinolide (**1a**), (2*R*,3*S*,22*R*,23*R*,24*S*)-2,3,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one, from the pollen of *Brassica napus*,[1] a number of synthetic isomers or analogues possessing in many cases high bioactivity have been reported.[2–7] Among those possessing significant bioactivity the (22*S*,23*S*,24*R*)-diastereoisomer (24*R*)-(**1b**), and both (2*R*,3*S*,22*R*,23*R*)- and (2*R*,3*S*,22*S*,23*S*)-2,3,22,23-tetrahydroxy-B-homo-7-oxa-5α-stigmastan-6-one (**1c**) and (**1d**), are worthy of mention. We now report the synthesis of (2*R*,3*S*,22*S*,23*S*)-2,3,22,23-tetrahydroxy-B-homo-7-aza-5α-stigmastan-6-one (**2a**), an aza-analogue of (**1d**), which might possess some hormonal activity.

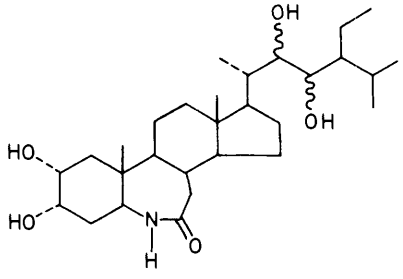
First attempts to obtain lactam (**2a**) by Beckmann rearrangement of the appropriate hydroxyimino steroid, in our



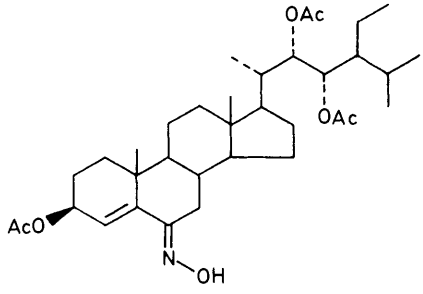
(**1**) **a**; 22*R*, 23*R*; R = Me
b; 22*S*, 23*S*; R = Me
c; 22*R*, 23*R*; R = Et
d; 22*S*, 23*S*; R = Et



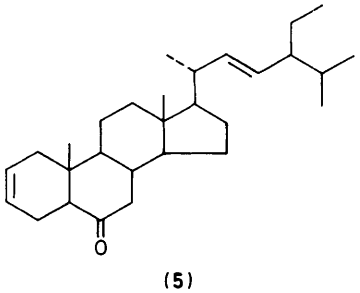
(**2**) **a**; 22*S*, 23*S*
b; 22*R*, 23*R*



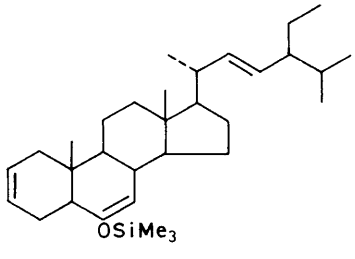
(**3**) **a**; 22*S*, 23*S*
b; 22*R*, 23*R*



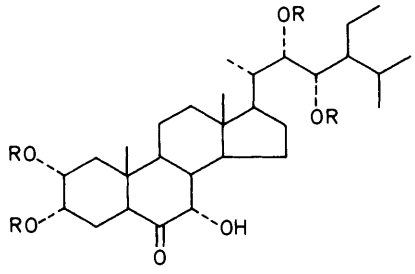
(**4**)



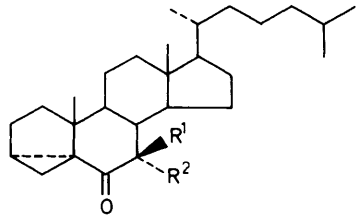
(**5**)



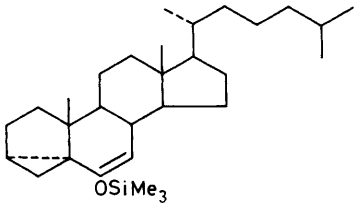
(**6**)



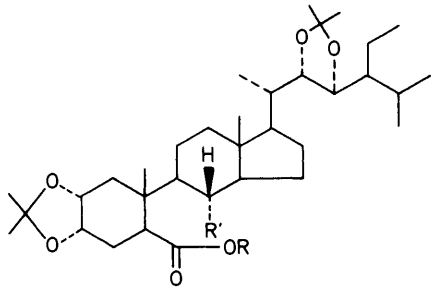
(7) **a**; R = H
b; RR = CMe_2



(8) **a**; R^1 = H, R^2 = $OSiMe_3$
b; R^1 = $OSiMe_3$, R^2 = H

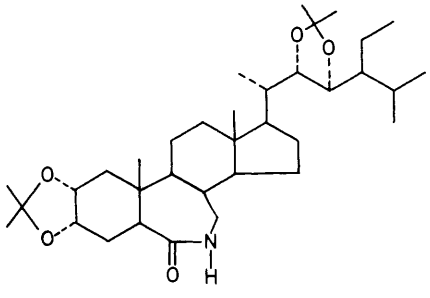


(9)



(10) **a**; R = H, R' = CHO
b; R = Me, R' = CHO
c; R = Me, R' = CH_2OH
d; R = Me, R' = CHNOH
e; R = Me, R' = CH_2NH_2
f; R = Me, R' = CH_2Mes
g; R = Me, R' = CH_2N_3

Mes = mesyl



(11)

and other laboratories,[8,9] were unsuccessful, since Beckmann rearrangement occurred in a highly regioselective way and afforded the 6-aza-B-homosteroids (**3a**) and (**3b**).

Unsuccessful results were also obtained in the Schmidt reaction of 6-oxo steroids under a variety of conditions,[8,9] and starting with (3*S*,22*S*,23*S*)-3,22,23-triacetoxy-6-hydroxyiminostigmast-4-ene (**4**) whose Δ^4 double bond did not reverse the regioselectivity of the Beckmann rearrangement.[10]* The successful route to the lactam (**2a**) started with 5α-stigmasta-2,22-dien-6-one (**5**) which derives from stigmasterol.[8] Enolsilylation of the dienone (**5**) under kinetic control of the reaction conditions afforded crystalline 6-trimethylsiloxy-5α-stigmasta-2,6,22-triene (**6**), the ^{1}H n.m.r. spectrum of which clearly shows the presence of five vinylic protons in the molecule. Treatment of silyloxy triene (**6**) with 3 mol equiv. of osmium tetraoxide in pyridine afforded (2*R*,3*S*,7*S*,22*S*,23*S*)-2,3,7,22,23-pentahydroxy-5α-stigmastan-6-one (**7a**). Compound (**7a**) showed the correct elemental analysis and physico-chemical properties and reactivity in agreement with the proposed structure. The wavenumber of its carbonyl peak at 1 725 cm^{-1} in the i.r. spectrum can be accounted for by a hydroxy group 'α' to the carbonyl group.[11] This is formed, as expected,[12] by action of osmium tetraoxide on the silyl enol ether group of compound (**6**).

The configuration of the 7-hydroxy group was derived from the shape of the 7β-proton signal in the ^{1}H n.m.r. spectrum of the (2*R*,3*S*,7*S*,22*S*,23*S*)-7-hydroxy-2,3;22,23-di-isopropylidenedioxy-5α-stigmastan-6-one (**7b**). It resonates as a broad singlet at δ 3.78 ($w_{\frac{1}{2}}$ 3.0 Hz), as expected for the coupling of the equatorial 7β-proton with its axial 8β-neighbour. A 7α-axial proton should display a one-proton doublet with a larger (9—11 Hz) coupling constant arising from its coupling with the 8β-axial proton.

These differences were indeed observed in the ^{1}H n.m.r. spectra of model compounds (**8a** and **b**) whose unsubstituted ring *A* and side-chain simplify the ^{1}H n.m.r. spectra.

Compound (**8a**) was obtained by osmylation and silylation of 6-trimethylsiloxy-3α,5-cyclo-5α-cholest-6-ene (**9**). In the ^{1}H n.m.r. spectrum of compound (**8a**) the signal attributed to the 7β-proton appears as a broad singlet at δ 3.78 ($w_{\frac{1}{2}}$ 3.0 Hz). Compound (**8b**) was obtained in mixture (2:3) with (**8a**) in a controlled ozonolysis of (**9**) using Sudan III as end-point indicator.[13] In the ^{1}H n.m.r. of compound (**8b**) the 7α-proton resonates as a sharp doublet centred at δ 3.80 (*J* 9.6 Hz).

The configuration of the 2- and 3-hydroxy groups of compound (**7a**), as well as the configuration of the hydroxy groups in the side-chain, were first assigned considering the well known high stereoselectivity of osmium tetraoxide oxidation of the Δ^2- and Δ^{22}-double bonds of stigmasterol.[8,14,15] The assignment of the 2*R*,3*S*,22*S*,23*S*-hydroxy group configurations was then confirmed by the successive transformation of compound (**7a**) into known[16] (2*R*,3*S*,22*S*,23*S*)-2,3,22,23-tetrahydroxy-B-homo-7-oxa-5α-stigmastan-6-one (**1d**). In order to accomplish the synthesis of lactam (**2a**), the pentahydroxy ketone (**7a**) was transformed into the diacetonide

* The results of a study on the Beckmann rearrangement of steroidal Δ^4-6-hydroxyimino compounds are reported in the accompanying paper.

(**7b**), which was oxidized with periodic acid to give (2*R*,3*S*,22*S*,23*S*)-2,3;22,23-di-isopropylidenedioxy-7-oxo-6,7-seco-5α-stigmastan-6-oic acid (**10a**), an amorphous material which resisted all efforts at crystallization, and which on esterification afforded a crystalline aldehydo ester (**10b**).

Epimerization at C-8 of compound (**10b**) during its formation by periodic acid oxidation of the acyloin (**7b**) was excluded when, in order to confirm the stereochemistry of the 2,3,22,23 carbon centres, we transformed the aldehydo ester (**10b**) into the known[16] homobrassinosteroid (**1d**) *via* sodium borohydride reduction to hydroxy ester (**10c**) and acidic treatment of the crude reaction product. Reductive amination of the aldehyde group of compound (**10b**) using hydrogen in the presence of Raney nickel, or nickel deposited on alumina, as catalyst,[17] even under severe conditions (100 °C; 700 lb in^{-2}), afforded the desired (2*R*,3*S*,22*S*,23*S*)-2,3;22,23-di-isopropylidenedioxy-B-homo-7-aza-5α-stigmastan-6-one (**11**) in low yields (*ca.* 10%). Thus we decided to effect the reductive amination of the aldehydic group of compound (**10b**) by catalytic reduction[18] of the corresponding hydroxyimino derivative (**10d**). In the presence of Adams' catalyst the reduction occurred under mild conditions (room temp.; 86 lb in^{-2}) to afford directly the lactam (**11**) in nearly quantitative yield. The same lactam (**11**) was obtained by cyclization of the amino ester (**10e**), obtained by reduction of compound (**10b**) with sodium cyanohydridoborate in the presence of ammonium acetate.[19,*] The lactam (**11**) showed physicochemical properties in good agreement with the assigned structure. However, in order to exclude the epimerization of the aldehydic group of compound (**10b**) during the formation of the hydroxyimino derivative (**10d**) or the reductive amination of (**10b**), independent structural proof was desirable. Thus we prepared the lactam (**11**) by heating the amino ester (**10e**), obtained from the 8*S*-alcohol (**10c**) *via* the mesyl ester (**10f**) and the azido ester (**10g**). The properties of the obtained compound were identical with those of compound (**11**) obtained above.

Hydrolysis of the acetal functions of lactam (**11**) accomplished the synthesis of the title compound (**2a**).

Biological testing of compound (**2a**) as plant-growth promotor, and an independent synthesis of (2*R*,3*S*,22*R*,23*R*)-2,3,22,23-tetrahydroxy-B-homo-7-aza-5α-stigmastan-6-one (**2b**), are in progress.

Experimental

All m.p.s are uncorrected. I.r. spectra were recorded for solutions in chloroform or for Nujol mulls on a Perkin Elmer 1420 spectrometer. ^{1}H N.m.r. spectra were recorded on a Varian EM-360L or on a Varian XL-200 instrument for solutions in $CDCl_3$ or in CD_3OD and are reported in δ units relative to Me_4Si. Optical rotations were measured for chloroform solutions. U.v. spectra were recorded for ethanolic solutions on a JASCO UVIDEC 430 A spectrophotometer. The progress of all reactions and column chromatography (Silica 60, 230—400 mesh) was monitored by t.l.c. on silica gel HF_{254} microplates. Hexane–ethyl acetate and dichloromethane–acetone mixtures were used as developing solvents, and spots were detected by spraying with 70% sulphuric acid, followed by heating. The usual work-up refers to dilution with water, extraction with an organic solvent, washing the extract to neutrality, drying (Na_2SO_4), and removal of the solvent under reduced pressure. Mass spectra were recorded on a Varian 112 S mass spectrometer (direct inlet).

6-Trimethylsiloxy-5α-stigmasta-2,6,22-triene (**6**).—The dienone (**5**) (2 g) was dissolved in tetrahydrofuran (THF) (20 ml) and the solution was added to a stirred solution of lithium di-isopropylamide at −78 °C, prepared by the addition of a solution of n-butyl-lithium in n-hexane (5.4 ml of a 1.6M solution) to a solution of di-isopropylamine (1.3 ml) in THF (20 ml) at −78 °C. After 1 h the resulting enolate solution was treated, at −78 °C, with a mixture of trimethylchlorosilane (1.2 ml) and triethylamine (0.5 ml), and the mixture was stirred for 15 min and then allowed to come to room temperature. After 1 h the mixture was filtered through a pad of Celite, and the filtrate was evaporated under reduced pressure. After the usual work-up the residue was crystallized from propan-2-ol to give the *trimethylsiloxy triene* (**6**) (2.1 g), m.p. 102—104 °C; δ 0.16 (9 H, s), 4.72 (1 H, br s, $w_{\frac{1}{2}}$ 3 Hz, 7-H), 5.12 (2 H, m, 22- and 23-H), and 5.66 (2 H, m, 2- and 3-H) (Found: C, 79.75; H, 11.2%; M^+ 482. $C_{32}H_{54}OSi$ requires C, 79.6; H, 11.3%; *M*, 482).

(2R,3S,7S,22S,23S)-2,3,7,22,23-*Pentahydroxy-5α-stigmastan-6-one* (**7a**).—A solution of osmium tetraoxide (2 g) in dry pyridine (20 ml) was added to a solution of the trimethylsiloxy triene (**6**) (1.15 g) in pyridine (20 ml). The mixture was stirred for 70 h in the dark. The solvent was removed under reduced pressure, the residue was dissolved in a mixture of methanol–dichloromethane (100 ml; 1:1 v/v), and hydrogen sulphide was bubbled through the solution for 7 h. The black precipitate was filtered off on a pad of Celite and washed with methanol–dichloromethane. Removal of the solvent and rapid chromatography of the residue afforded the *pentahydroxy ketone* (**7a**) (900 mg), m.p. 92—95 °C (amorphous); $\nu_{max.}$ 3 420, 1 725, 1 480, and 1 400 cm^{-1}; δ 3.5—4.1 (5 H, overlapping, 2-, 3-, 7-, 22-, and 23-H) (Found: C, 70.5; H, 10.3. $C_{29}H_{50}O_6$ requires C, 70.4; H, 10.2%).

From the last fractions of the chromatography a product (80 mg) was obtained whose elemental analysis agrees with the molecular formula $C_{29}H_{50}O_6$.

(2R,3S,7S,22S,23S)-7-*Hydroxy*-2,3;22,23-*di-isopropylidenedioxy-5α-stigmastan-6-one* (**7b**).—To a solution of the pentahydroxy ketone (**7a**) (1 g) and 2,2-dimethoxypropane (10 ml) in dichloromethane (10 ml) was added a trace of toluene-*p*-sulphonic acid (PTSA), and the mixture was stirred for 2 h at 0 °C, then poured into saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane. Usual work-up, and crystallization from methanol, afforded the *7-hydroxy ketone* (**7b**) (950 mg), m.p. 217—218 °C; $\nu_{max.}$ 1 720 cm^{-1}; δ 0.63 (3 H, s, 19-H_3), 0.69 (3 H, s, 18-H_3), 0.95 (3 H, d, *J* 6.6 Hz), 0.96 (3 H, d, *J* 6.8 Hz), 0.97 (3 H, t, *J* 3.7 Hz), 1.03 (3 H, d, *J* 6.8 Hz), 1.34 (3 H, s), 1.37 (6 H, s), 1.50 (3 H, s), 2.53 (1 H, dd, *J* 4.1 and 8.5 Hz, 5-H), 3.78 (1 H, br s, $w_{\frac{1}{2}}$ 3.0 Hz, 7β-H), 3.90 (1 H, dd, *J* 3.2 and 8.5 Hz), 3.99 (1 H, dd, *J* 2.2 and 8.5 Hz) and 4.10—4.45 (2 H, m, overlapping, 2- and 3-H); *m/z* 559 (M^+ − 15, 20%), 489 (27), 445 (37), 387 (100), and 359 (43) (Found: C, 73.2; H, 10.3. $C_{35}H_{58}O_6$ requires C, 73.1; H, 10.2%).

Methyl (2R,3S,22S,23S)-2,3;22,23-*Di-isopropylidenedioxy*-7-*oxo*-6,7-*seco-5α-stigmastan-6-oate* (**10b**).—The hydroxy ketone (**7b**) (500 mg) was dissolved in diethyl ether (80 ml) and treated at 0 °C with periodic acid dihydrate (250 mg). After being stirred for 1 h, the mixture was allowed to come to room temperature and was stirred for another 3 h and then filtered, and the solid was washed with diethyl ether. Usual work-up, followed by rapid chromatography, afforded the (2R,3S,22S,23S)-2,3;22,23-*di-isopropylidenedioxy*-7-*oxo*-6,7-*seco-5α-stigmastan-6-oic acid* (**10a**) (410 mg) as an amorphous material, m.p. 115—117 °C (sintered at 92—94 °C); $\nu_{max.}$ 3 200—2 700, 2 820, 2 730, 1 760,

* This work was presented at the XI Conference on Isoprenoids, Jachranka, Poland, 21—26 September 1985. On this occasion Professor K. Mori told us that he had obtained the lactam (**2a**) by reductive amination of the aldehyde acid (**10a**) with $NaBH_3CN$ in the presence of ammonium acetate, followed by cyclization.

1 725, and 1 710 cm^{-1} (Found: C, 71.2; H, 10.0. $C_{35}H_{58}O_7$ requires C, 71.15; H, 9.9%).

Treatment of the aldehydo acid (**10a**) (300 mg) with diazomethane in diethyl ether at 0 °C afforded the corresponding *methyl ester* (**10b**) (250 mg), m.p. 127—128 °C (from methanol); $\nu_{max.}$ 2 820, 2 730, 1 735, and 1 725 cm^{-1}; δ 1.3 (3 H, s), 1.4 (6 H, s), 1.5 (3 H, s), 2.9 (1 H, dd, *J* 5.0 and 9.0 Hz, 5-H), 3.7 (3 H, s, CO_2Me), 3.9—4.0 (2 H, m, overlapping, 22- and 23-H), 4.1—4.4 (2 H, m, overlapping, 2- and 3-H), and 9.45 (1 H, d, *J* 5.0 Hz); *m/z* 589 ($M^+ - 15$, 100%), 561 (14), 519 (74), 475 (66), and 447 (74) (Found: C, 71.6; H, 9.9. $C_{36}H_{60}O_7$ requires C, 71.5; H, 10.0%).

(2R,3S,22S,23S)-2,3,22,23-*Tetrahydroxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (**1d**).—A solution of the aldehydo ester (**10b**) (1 g) in a mixture of methanol (100 ml) and diethyl ether (20 ml) was treated at 0 °C with a solution of sodium borohydride (100 mg) in propan-2-ol (10 ml) for 30 min to afford, after work-up, *methyl* (2R,3S,22S,23S)-7-*hydroxy*-2,3;22,23-*di-isopropylidenedioxy*-6,7-*seco*-5α-*stigmastan*-6-*oate* (**10c**) (900 mg), m.p. 162—164 °C (from methanol); $\nu_{max.}$ 3 630, 3 500, and 1 725 cm^{-1}; δ 1.3 (3 H, s), 1.4 (6 H, s), 1.5 (3 H, s), 3.0 (1 H, dd, *J* 5.0 and 9.0, 5-H), 3.5—3.8 (5 H, m, overlapping, CO_2Me and 7-H_2), 3.9—4.0 (2 H, m, overlapping, 22- and 23-H), and 4.1—4.4 (2 H, m, overlapping, 2- and 3-H) (Found: C, 71.2; H, 10.3. $C_{36}H_{62}O_7$ requires C, 71.25; H, 10.3%). In order to avoid direct lactonization of hydroxy ester (**10c**) the extraction solvent must be eliminated at low temperature (under 20 °C).

Oxidation of compound (**10c**) (50 mg) with Sarett's reagent [20] afforded the aldehyde (**10b**) (45 mg).

When the hydroxy ester (**10c**) (800 mg) was refluxed in anhydrous toluene for 10 min, work-up afforded (2R,3S,22S,23S)-2,3;22,23-*di-isopropylidenedioxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (720 mg), m.p. 136—138 °C (from methanol); $\nu_{max.}$ 1 735 cm^{-1}; δ 0.70 (3 H, s), 0.83 (3 H, s), 1.34 (3 H, s), 1.37 (6 H, s), 1.50 (3 H, s), 3.27 (1 H, dd, *J* 6 and 10 Hz), 3.90—4.00 (2 H, m, overlapping, 22- and 23-H), 4.03—4.10 (2 H, m, overlapping, 7-H_2), and 4.15—4.40 (2 H, m, overlapping, 2- and 3-H) (Found: C, 73.2; H, 10.1%; M^+, 574. $C_{35}H_{58}O_6$ requires C, 73.1; H, 10.2%, *M*, 574).

This lactone (700 mg) was dissolved in methanol (150 ml) containing hydrochloric acid (7 ml; 10 M), and the solution was refluxed for 1 h to afford, after work-up, the tetrahydroxy lactone (**1d**) (580 mg), m.p. 206—208 °C (from methanol); $[\alpha]_D^{22}$ 37° {lit.,[16] 193—194 °C (from methanol); $[\alpha]_D^{20}$ 35.6°}; *m/z* 494 (M^+, 1%), 476 (1), 461 (2), 460 (2), 447 (1), 433 (1), 417 (1), 410 (7), 409 (29), 391 (8), 381 (45), 380 (100), 379 (66), 362 (60), 361 (66), 350 (90), 343 (47), 303 (33), 285 (30), and 208 (40) (Found: C, 70.5; H, 10.3. Calc. for $C_{29}H_{50}O_6$: C, 70.4; H, 10.2%). All the other physicochemical characteristics are identical with those reported by Mori *et al.*[16]

(2R,3S,22S,23S)-2,3;22,23-*Di-isopropylidenedioxy*-B-*homo*-7-*aza*-5α-*stigmastan*-6-*one* (**11**).—(a) *From methyl* (2R,3S,22S,23S)-7-*hydroxy*-2,3;22,23-*di-isopropylidenedioxy*-6,7-*seco*-5α-*stigmastan*-6-*oate* (**10c**). A solution of the hydroxy ester (**10c**) (500 mg) in dry dichloromethane (20 ml) containing triethylamine (1.5 ml) was treated at 0 °C with methanesulphonyl chloride (0.4 ml) for 1 h to afford, after work-up, the *mesyl ester* (**10f**) (540 mg): an oil; $\nu_{max.}$ 1 725, 1 360, 1 340, and 1 175 cm^{-1} (Found: C, 64.8; H, 9.4. $C_{37}H_{64}O_9S$ requires C, 64.9; H, 9.4%).

The mesyl derivative (**10f**) (500 mg) was dissolved in dimethylformamide (4 ml) and the solution was treated with a solution of sodium azide (140 mg) in water (0.7 ml). The mixture was refluxed for 4 h, when work-up and chromatography afforded the azido ester (**10g**) (400 mg), m.p. 120—121 °C (triturated in methanol); $\nu_{max.}$ 2 110 and 1 725 cm^{-1} (Found: C, 68.3; H, 9.6; N, 6.6. $C_{36}H_{61}N_3O_6$ requires C, 68.4; H, 9.7; N, 6.65%).

The azido ester (**10g**) (800 mg) was dissolved in ethyl acetate (200 ml) and hydrogenated in the presence of Raney Nickel to afford methyl (2R,3S,22S,23S)-7-amino-2,3;22,23-di-isopropylidenedioxy-6,7-seco-5α-stigmastan-6-oate (**10e**) (750 mg) as a glass, $\nu_{max.}$ 3 420, 3 240, and 1 725 cm^{-1}.

The crude amino ester (**10e**) was dissolved in xylene (commercial mixture; 30 ml) and the solution was refluxed for 1 h to afford, after elimination of the solvent under reduced pressure, and chromatography, (2R,3S,22S,23S)-2,3;22,23-*di-isopropylidenedioxy*-B-*homo*-7-*aza*-5α-*stigmastan*-6-*one* (**11**) (650 mg), m.p. 166—168 °C (from methanol); $\nu_{max.}$ 3 430, 1 660, 1 470, 1 385, 1 170, and 995 cm^{-1}; δ 0.70 (3 H, s), 0.88 (3 H, s), 0.95 (3 H, d, *J* 6.7 Hz), 0.96 (3 H, t, *J* 7.5 Hz), 0.97 (3 H, d, *J* 7.0 Hz), 1.03 (3 H, d, *J* 6.5 Hz), 1.31 (3 H, s), 1.35 (6 H, s), 1.53 (3 H, s), 2.27 (1 H, dd, *J* 3.5 and 16 Hz), 3.00 (1 H, ddd, *J* 2.5, 7.5, and 15 Hz, 7aα-H), 3.10 (1 H, dd, *J* 7 and 7.5 Hz, 5-H), 3.14 (1 H, ddd, *J* 5.5, 10, and 15 Hz, 7aβ-H), 3.87 (1 H, dd, *J* 3.5 and 8.5 Hz, 22-H), 3.97 (1 H, dd, *J* 2 and 8.5 Hz, 23-H), 4.28—4.44 (2 H, m, overlapping, 2- and 3-H), and 6.02 (1 H, dd, *J* 5.5 and 7.5 Hz, NH); *m/z* 573 (M^+, 0.7%), 558 (14), 515 (2), 498 (7), 488 (8), 444 (11), 4.18 (15), 389 (60), 372 (15), 360 (10), 326 (10), and 185 (100) (Found: C, 73.2; H, 10.4; N, 2.5. $C_{35}H_{59}NO_5$ requires C, 73.25; H, 10.3; N, 2.4%).

(b) *From methyl* (2R,3S,22S,23S)-7-*hydroxyimino*-2,3;22,23-*di-isopropylidenedioxy*-6,7-*seco*-5α-*stigmastan*-6-*oate* (**10d**). The *hydroxyimino ester* (**10d**) (200 mg), obtained by the usual procedure [m.p. 92—93 °C (amorphous); $\nu_{max.}$ 3 600, 1 725, 1 390, and 1 380 cm^{-1}; δ 1.3 (3 H, s), 1.35 (6 H, s), 1.5 (3 H, s), 3.0 (1 H, dd, *J* 7 and 7.5 Hz), 3.7 (3 H, s, CO_2Me), 3.9—4.0 (2 H, m, overlapping, 22- and 23-H), 4.1—4.4 (2 H, m, overlapping, 2- and 3-H), 7.1 (1 H, d, *J* 9 Hz, 7-H), and 8.1 (1 H, m, NOH) (Found: C, 69.7; H, 9.9; N, 2.25. $C_{36}H_{61}NO_7$ requires C, 69.75; H, 9.9; N, 2.3%)], was dissolved in acetic acid (30 ml) and hydrogenated in the presence of platinum dioxide (Adams' catalyst) (80 mg) in an autoclave at 25 °C and 86 lb in^{-2} for 6 h. Filtration and elimination of the acetic acid, followed by the usual work-up, afforded the lactam (**11**) (175 mg), m.p. 166—168 °C (from methanol), with physical and spectroscopic properties identical with those described above.

(c) *From methyl* (2R,3S,22S,23S)-2,3;22,23-*di-isopropylidenedioxy*-7-*oxo*-6,7-*seco*-5α-*stigmastan*-6-*oate* (**10b**). A solution of the aldehydo ester (**10b**) (500 mg), anhydrous ammonium acetate (5 g), and sodium cyanohydridoborate (500 mg) in a mixture of methanol (150 ml) and THF (30 ml) was stirred for 70 h at 25 °C. After removal of the solvent under reduced pressure, and the usual work-up (dichloromethane extraction), crude amino ester (**10e**) (450 mg) was obtained, identical (t.l.c., i.r.) with that obtained above. Lactamization as previously described afforded the lactam (**11**) (390 mg), identical with that obtained above.

(2R,3S,22S,23S)-2,3,22,23-*Tetrahydroxy*-B-*homo*-7-*aza*-5α-*stigmastan*-6-*one* (**2a**).—Treatment of the lactam (**11**) (400 mg) with methanol (80 ml) containing hydrochloric acid (4 ml; 10 M) at reflux for 1 h afforded (2R,3S,22S,23S)-2,3,22,23-*tetrahydroxy*-B-*homo*-7-*aza*-5α-*stigmastan*-6-*one* (**2a**) (310 mg), m.p. 157—160 °C (sintered) and 200—223 °C (from di-isopropyl ether); $\nu_{max.}$ (Nujol) 3 600, 3 100, and 1 665 cm^{-1}; δ(CD_3OD) 0.76 (3 H, s), 0.86 (3 H, d, *J* 6.7 Hz), 0.90 (3 H, s), 0.93 (3 H, d, *J* 6.8 Hz), 0.95 (3 H, t, *J* 7.3 Hz), 1.02 (3 H, d, *J* 6.8 Hz), 2.95—3.05 (3 H, m, overlapping, 5-H and 7a-H_2), 3.50—3.63 (3 H, m, overlapping), and 3.88—3.94 (1 H, m); *m/z* 493 (M^+, 1%), 475 (2), 460 (4), 459 (6), 408 (12), 390 (15), 380 (38), 379 (70), 378 (46), 361 (62), 360 (100), and 349 (38) (Found: C, 66.5; H, 10.1; N, 2.7. $C_{29}H_{51}NO_5 \cdot 1.5\,H_2O$ requires C, 66.9; H, 10.45; N, 2.7%).

Treatment of the tetrahydroxy lactam (**2a**) with 2,2-di-

methoxypropane in dichloromethane and a trace of PTSA afforded the di-isopropylidene derivative (**11**), with physical and spectroscopic properties identical with those described above.

7α-Trimethylsiloxy-3α,5-cyclo-5α-cholestan-6-one (**8a**).—A solution of 3α,5-cyclo-5α-cholestan-6-one (1 g),[21] in THF (10 ml) was added to a stirred solution of lithium di-isopropylamide at −78 °C, prepared by addition of a solution of butyl-lithium in hexane (2.9 ml; 1.6M) to a solution of di-isopropylamine (0.7 ml) in THF (10 ml) at −78 °C. After 1 h the resulting enolate solution was treated at −78 °C with a mixture of trimethylchlorosilane (0.7 ml) and triethylamine (0.3 ml), stirred for 15 min, and then allowed to come to room temperature. After 1 h the mixture was filtered over a pad of Celite, and the filtrate was concentrated under reduced pressure. Work-up afforded 6-trimethylsiloxy-3α,5-cyclo-5α-cholest-6-ene (**9**) (1 g) as a glass, δ 4.7 (1 H, br s, $w_{\frac{1}{2}}$ 3 Hz, 7-H).

A solution of silyl enol ether (**9**) (900 mg) in pyridine (25 ml) was treated with osmium tetraoxide (600 mg) at room temperature for 12 h in the dark. Elimination of pyridine, reduction (H_2S) of the osmate esters, and usual work-up (dichloromethane extraction) afforded a crude product (900 mg), which was dissolved in triethylamine (30 ml) and treated with trimethylchlorosilane (1 ml) to afford, after work-up, the *siloxy ketone* (**8a**) (830 mg), m.p. 88—89 °C (from methanol); $\nu_{max.}$ 1 700 cm^{-1}; δ 3.78 (1 H, br s, $w_{\frac{1}{2}}$ 3.0 Hz) (Found: C, 76.2; H, 11.2%; M^+, 472. $C_{30}H_{52}O_2Si$ requires C, 76.2; H, 11.1%; *M*, 472).

7β-Trimethylsiloxy-3α,5-cyclo-5α-cholestan-6-one (**8b**).—The silyl enol ether (**9**) (700 mg) was dissolved in dichloromethane (50 ml) containing pyridine (1 ml) and Solvent Red 23 (Sudan III) (3 mg) and was ozonized at −78 °C until the dye had been totally bleached. Then the reaction vessel was flushed with nitrogen for 15 min and excess of tris(diethylamino)phosphine was added, and the solution was allowed to warm up to room temperature. Usual work-up, followed by column chromatography, afforded a 3:2 mixture (^1H n.m.r. and g.l.c.) of epimeric 7-siloxy-6-ketones (**8a** and **b**). The epimers (**8a** and **b**) showed the same mobility on t.l.c. in different mixtures of developers. In contrast they had different retention times in g.l.c. analysis [240 °C: (**8a**), R_t (rel.) 1.3; (**8b**) R_t (rel.) 1]. By preparative g.l.c. a few milligrams of pure (**8b**) were obtained, which showed m.p. 93—95 °C (from methanol); $\nu_{max.}$ 1 690 cm^{-1}; δ 3.80 (1 H, d, *J* 9.6 Hz) (Found: C, 76.3; H, 11.6%; M^+, 472. $C_{30}H_{52}O_2Si$ requires C, 76.2; H, 11.1%; *M*, 472).

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