Stereoselective Synthesis of Plant Growth-promoting Steroids, Brassinolide, Castasterone, Typhasterol, and Their 28-Nor Analogues

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Plant growth-promoting steroids, brassinolide (1a), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one, castasterone (2a), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one, 28-norbrassinolide (1b), (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-cholestan-6-one, brassinone (2b), (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-5α-cholestan-6-one, and typhasterol (2c), (22*R*,23*R*,24*S*)-3α,22,23-trihydroxy-5α-ergostan-6-one, have been stereoselectively synthesized. These steroids show very strong biological activities in three different kinds of bioassays.

Brassinolide (1a), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one, isolated from the pollen of rape (*Brassica napus* L.) is a new plant growth hormonal steroid having an unprecedented seven-membered B-ring lactone and four successive asymmetric centres in the side-chain.[1] Brassinolide (1a) promotes cell elongation, cell division, and plant growth at very low concentration,[1] and also showed a wide variety of responses in a number of bioassays for auxin, gibberellin, and cytokinin.[2-6] Therefore, this steroid or its related compounds will find practical application in agriculture.[7] Because of the scarcity, the remarkable biological activities and the novel structural features, much effort has been made to synthesize brassinolide (1a). We have briefly reported the first stereoselective synthesis of brassinolide (1a).[8] Three other groups have also succeeded in synthesizing this fascinating steroid.[9-11] After the synthesis of brassinolide (1a), a number of its related compounds were synthesized and their plant growth-promoting activities were investigated.[12-22]

Subsequent to the isolation of brassinolide (1a), castaterone (2a), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one, was isolated from the insect galls of the chestnut tree (*Castanea spp.*) as a plant growth promoter.[23] Recently, very small amounts of new brassinolide analogues (22*R*,23*R*)-28-norbrassinolide (1b), (22*R*, 23*R*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-cholestan-6-one, and brassinone (2b), (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-5α-cholestan-6-one, were identified together with brassinolide (1a) and castasterone (2a), in the immature seeds and sheaths of chinese cabbage (*Brassica campestris var. Pekinensis*), the leaves of green tea (*Thea sinensis*) and *Distylium racemosum* Sieb et Zucc., and the insect galls of chestnut tree and *Distylium racemosum*.[24-28] Much more recently, typhasterol (2c), (22*R*,23*R*,24*S*)-3α,22,-23-trihydroxy-5α-ergostan-6-one, was isolated from cat-tail pollen (*Typha latifolia* L.) as the first example of a plant growth hormonal 2-deoxy steroid.[29] In the present paper we describe details of our synthesis of brassinolide (1a), castasterone (2a), typhasterol (2c), and their 28-norsteroids (1b) and (2b).

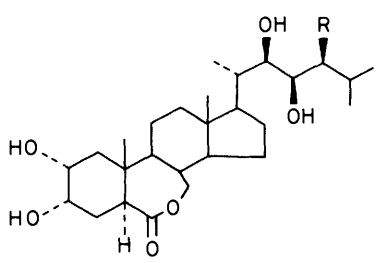
We assumed that functionalization at ring A and B for brassinolide (1a) could be easily achieved *via* the 2α,3α,22,23-tetra-acetoxy-5α-ergostan-6-one (17), since it was reported that Baeyer-Villiger oxidation of 3β-acetoxy-5α-cholestan-6-one gave the desired 7-oxalactone, 3β-acetoxy-B-homo-7-oxa-5α-cholestan-6-one as a major product.[30,31] Indeed, as a model experiment, 2α,3α-diacetoxy-5α-cholestan-6-one was oxidized with peracid to give the desired 2α,3α-diacetoxy-B-homo-7-oxa-5α-cholestan-6-one with *ca.* 90% regioselectivity. Therefore, this reaction is a key one in forming the 7-oxalactone moiety of brassinolide (1a).

The most important problem for the synthesis of brassinolide (1a) is the construction of the side chain of (1a). According to our previous investigation into the stereochemistry of the electrophilic reactions at the C-22(23) double bond,[32,33] direct *cis*-hydroxylation of the side chain of (22*E*,24*S*)-22-dehydrocampesterol with osmium tetraoxide should be an impractical approach to the preparation of the side chain of (1a) because it is likely that the unnatural (22*S*,23*S*)-vicinal diol isomer will be obtained as a major product. Thus, we completed the synthesis of the side chain using an alternative method; namely, introduction of asymmetry at C-22 at first, which in turn controls the stereochemistry of C-23 and C-24 by hydroxy-directing epoxidation,[34] and finally introduction of a methyl group at the C-24 position.

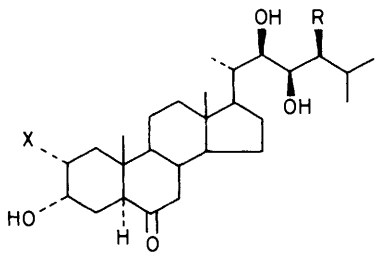
The 22-aldehyde (3) [35] derived from commercially available dinorcholenic acid was treated with 3-methylbut-1-ynyllithium in tetrahydrofuran at −78 °C to give a 3 : 2 epimeric mixture of the 22-alcohols, from which the major (22*R*)-isomer (5) was isolated by recrystallization from methanol in 38% yield. The configuration at C-22 of (5) was confirmed by conversion of (5) into the known (22*S*)-22-hydroxycholesterol (6a) and its diacetate derivative (6b).[36] The (22*S*)-alcohol (4) obtained from the mother liquor by recrystallization was submitted to methanesulphonation followed by treatment with potassium superoxide in dimethylformamide and dimethyl sulphoxide in the presence of 18-crown-6 to provide the desired 22*R*-alcohol (5) in 65% yield.

The acetylenic alcohol (5) was partially hydrogenated using Lindlar catalyst to give the *cis*-allylic alcohol (7) in 97% yield. Epoxidation of (7) with t-butyl hydroperoxide in the presence of a catalytic amount of oxovanadium acetylacetonate in benzene provided the (22*R*,23*R*,24*R*)-epoxy alcohol (8) in 85% yield as a single product according to the mechanism proposed by Sharpless.[34] The ^{1}H n.m.r. spectrum of (8) also supported its configuration at the C-23 position ($CDCl_3$: δ 3.07, dd, *J* 8 and 4 Hz, 23-H). At this stage direct introduction of a methyl group at the C-24 position with inversion was attempted using a number of reagents, Me_2CuLi, MeMgI, MeMgI/CuI, MeLi/HMPA, and Me_3Al, *etc.*, only to fail in getting the desired 24-methylated compound.[11b]

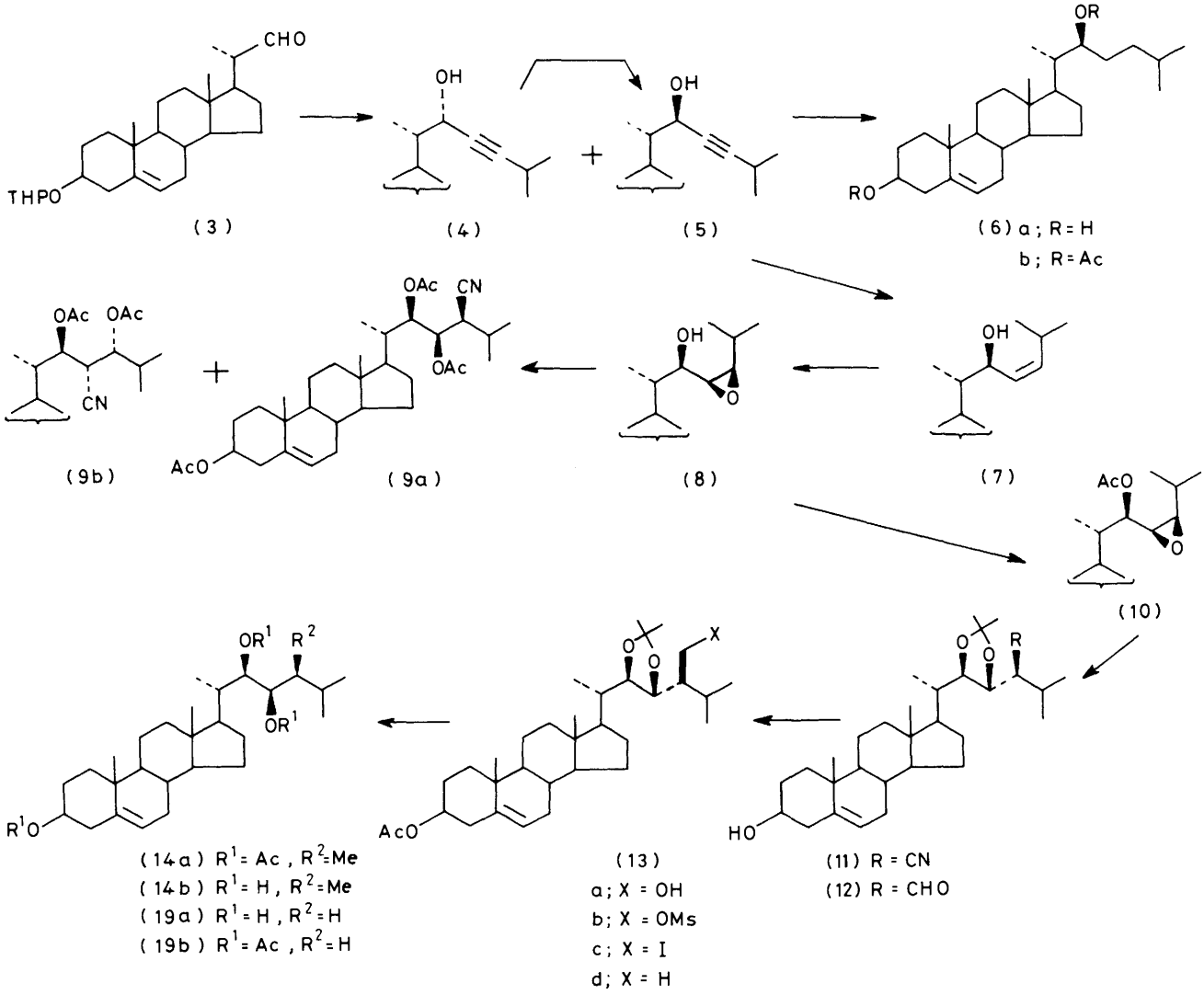
Consequently, we turned to the introduction of a cyano group at the C-24 position, which could then be converted into a methyl group. Although attempted introduction of a cyano group using KCN and 18-crown-6 failed, treatment of the epoxy alcohol (8) with hydrogen cyanide and triethylaluminium in tetrahydrofuran provided, after removal of tetrahydropyranyl group and acetylation, the desired 24-nitrile (9a) and the regioisomeric 23-nitrile (9b) in a ratio of 2 : 1. Thus we assumed that hydrocyanation of the epoxy acetate (10) would preferentially give the desired 24-cyano compound as a result of steric hindrance and the electronic inductive effect of the acetyl group. The acetate (10) was treated with an excess of



(1)
a; R = Me
b; R = H



(2)
a; R = Me , X = OH
b; R = H , X = OH
c, R = Me , X = H

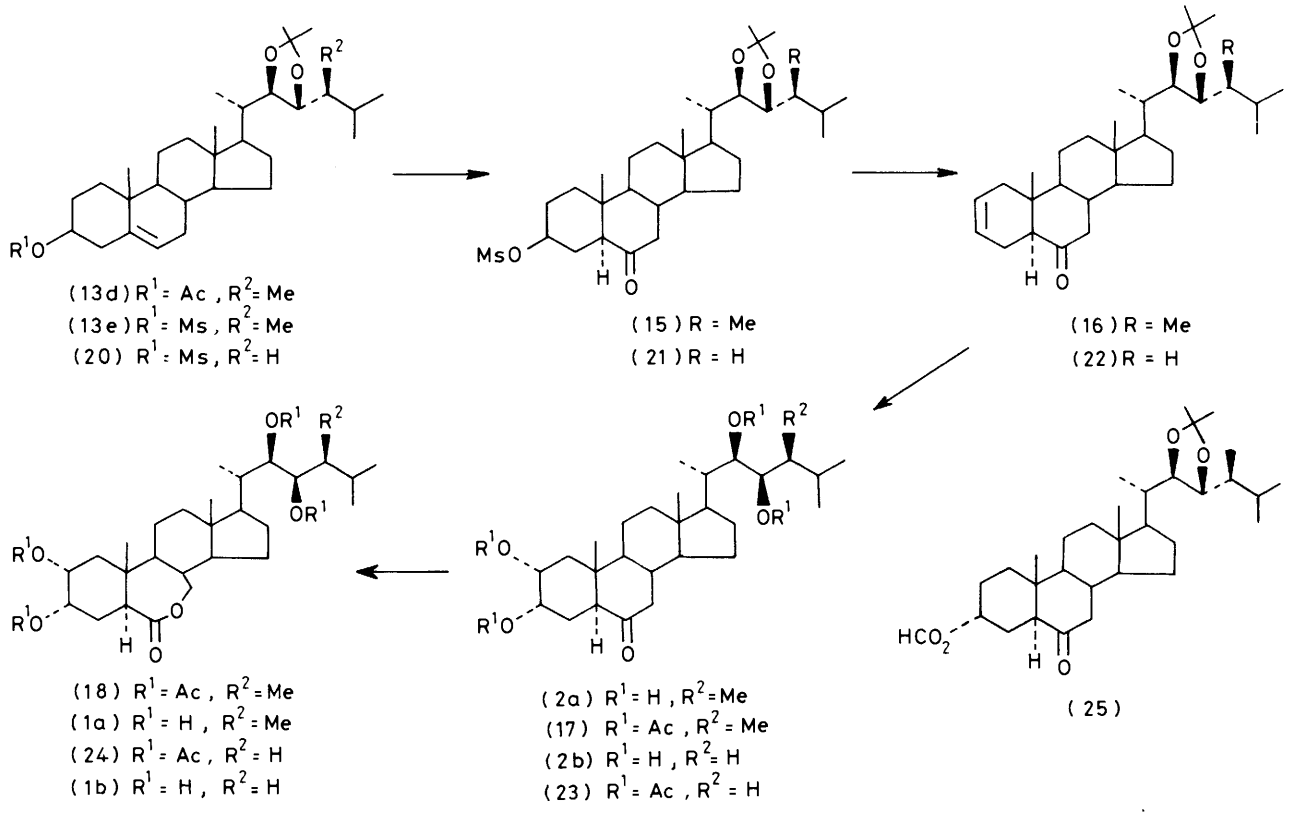


(3) (4) (5) (6) a; R = H
b; R = Ac
(7) (8) (9a) (9b) (10)
(11) R = CN
(12) R = CHO
(13)
a; X = OH
b; X = OMs
c; X = I
d; X = H
(14a) R^1 = Ac , R^2 = Me
(14b) R^1 = H , R^2 = Me
(19a) R^1 = H , R^2 = H
(19b) R^1 = Ac , R^2 = H

hydrogen cyanide–triethylaluminium and the product saponified; removal of the tetrahydropyranyl group followed by acetonide formation, afforded the desired 24-cyano compound (11) in 56% overall yield. The ^{13}C n.m.r. spectrum of (11) indicated that it was a single compound.* The configuration at C-24 of the nitrile (11) can be assigned as *S* by the established *trans* ring opening of the epoxide on hydrocyanation.[39] This was finally confirmed by transformation of (11) into brassinolide (1a) itself.

Transformation of the cyano group into a methyl group was accomplished as follows. Reduction of the nitrile (11) with di-isopropylaluminium hydride provided the aldehyde (12) in 65% yield. Acetylation of (12) and reduction with sodium borohydride gave the 28-alcohol (13a), which was converted into the mesylate (13b). This was treated with sodium iodide

* ^{13}C N.m.r. spectroscopy is effective for distinguishing the C-24 stereoisomers of C-24 substituted steroids.[38]



in acetone under reflux to yield the iodide (13c), which upon reduction with tributyltin hydride in tetrahydrofuran afforded the acetate (13d) in 77% overall yield. The acetonide (13d) was refluxed with 70% aqueous acetic acid and saponified to provide (22*R*,23*R*,24*S*)-3β,22,23-trihydroxyergost-5-ene (14b), which has the same side chain as brassinolide (1a).

Functionalization of the triol (14b) at rings A and B was carried out as follows. The acetate (13d) was converted into the mesylate (13e), which upon hydroboration with an excess of BH_3–THF complex in tetrahydrofuran and alkaline H_2O_2 oxidation, followed by treatment with pyridinium chlorochromate afforded the 6-oxo steroid (15). Treatment of (15) with lithium bromide in dimethylformamide under reflux [40] provided, after chromatographic purification, (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy-5α-ergost-2-ene-6-one (16) in 65% yield from (13d). Stereospecific α-face hydroxylation [41] of the 2-ene (16) was effected with a catalytic amount of osmium tetraoxide in a mixture of Bu^tOH–THF–H_2O (10 : 3 : 1) in the presence of *N*-methylmorpholine *N*-oxide.[42] Treatment of the resulting 2α,3α-diol with 70% aqueous acetic acid under reflux and recrystallization from ethyl acetate provided castasterone (2a), m.p. 250—252 °C (lit.,[10] m.p. 250—251 °C). Acetylation of this tetraol (2a) with acetic anhydride and pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine at 60 °C overnight afforded the castasterone tetra-acetate (17) in 80% yield from the 2-ene (16). This 6-oxo steroid (17) was submitted to Baeyer-Villiger oxidation.* Treatment of (17) with an excess of trifluoroperacetic acid in dichloromethane in the presence of disodium hydrogen phosphate at 0 °C for 3 h and chromatographic purification provided the brassinolide tetra-acetate (18) in 85% yield.* In its ^1H n.m.r. spectrum, the 7-oxalactone (18) exhibited signals due to 5α-H [$CDCl_3$; δ 3.00 (dd, *J* 13 and 6 Hz) and 7-H_2 (δ 4.10, m)] which are characteristic of this kind of 7-oxalactone.[31,43] The tetra-acetate (18) was saponified with 5% aqueous potassium hydroxide in methanol under reflux and subsequently acidified with concentrated HCl to afford brassinolide (1a), m.p. 273—278 °C (lit.,[1] m.p. 274—275 °C), $[\alpha]_D$ +16.0° (*c* 0.985, CH_2Cl_2–MeOH, 1 : 1). The i.r. ^{13}C n.m.r. and mass (EI) spectra of our synthetic brassinolide (1a) were identical with those of natural brassinolide (1a), which were kindly sent to us by Dr. M. D. Grove.

We then turned our attention to the synthesis of (22*R*,23*R*)-28-norbrassinolide (1b) and its 6-oxo steroid (2b). The key intermediate, (22*R*,23*R*)-22,23-dihydroxycholesterol (19a) was prepared from the epoxy alcohol (8) as follows. The methoxymethyl ether of (8) was reduced with lithium aluminium hydride and subsequently acetylated. Removal of the protecting groups with acid and then base provided the triol (19a) in 70% yield. According to the method described for the synthesis of brassinolide (1a), this triol (19a) was transformed, *via* (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-5α-cholestan-6-one (2b), m.p. 254—255 °C, into (22*R*,23*R*)-28-norbrassinolide (1b), m.p. 256—259 °C, $[\alpha]_D$ +32.0° (*c* 1.15, MeOH).

We next synthesized typhasterol (2c). Treatment of the 3β-methylsulphonyloxy-6-oxo steroid (15) with lithium carbonate instead of lithium bromide in dimethylformamide at 130 °C provided the 3α-formate (25) [26%, ^1H n.m.r. ($CDCl_3$): δ 5.23 (1 H, m, $W_{\frac{1}{2}}$ = 7 Hz, 3β-H), 8.01 (1 H, s, formyl)] and the 2-ene (16) (51%). Refluxing of (25) with aqueous acetic acid and subsequent saponification afforded typhasterol (2c), m.p. 225—228.5 °C (lit.,[29] m.p. 227—230 °C), in 85% yield. The ^1H n.m.r. spectrum (360 MHz) of our synthetic (2c) was identical with that of natural typhasterol (2c), which was kindly sent to us by Dr. K. Yoshihara. The synthesis of typhasterol (2c) firmly confirmed the assigned structure of typhasterol (2c).

Our synthetic brassinolide (1a), castasterone (2a), and their 28-nor analogues (1b) and (2b) exhibited very strong plant growth-promoting activities in the rice-lamina inclination test, Raphanus test, and Tomato test at a range of concentration of

* A detailed investigation into the Baeyer-Villiger oxidation of 5α-cholestan-6-one derivatives has been reported.[43]

1.0—0.0001 p.p.m.[6,17,18] Biological activities of typhasterol (2c) and its related 2-deoxysteroids are now under investigation and will be reported in due course.

Experimental

M.p.s were determined with a hot-stage microscope and are uncorrected. I.r. spectra were taken with a Hitachi Model 260-10 spectrometer. Optical rotations were determined with a CARL ZEISS LEP A-1 polarimeter. N.m.r. spectra were recorded on a JEOL PS-100 spectrometer in $CDCl_3$ solution with tetramethylsilane as an internal standard, unless otherwise stated. Mass spectra were taken on a Shimadzu LKB-9000S, a Shimadzu GC-MS 6020 or a Hitachi M-80 mass spectrometer. Column chromatography was normally effected with Kieselgel $60F_{254}$ (70—230 mesh, Merck). Kieselgel 60 F_{254} (0.25 mm thickness, Merck) was used for analytical and preparative thin-layer chromatography. Work-up refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying over magnesium sulphate, filtration, and removal of the solvent under reduced pressure. Ether refers to diethyl ether, THF to tetrahydrofuran, and THP to tetrahydropyranyl.

(22S)- *and* (22R)-3β-*Tetrahydropyranyloxycholest*-5-*en*-23-*yn*-22-*ol* (4) *and* (5).—To the solution of n-butyl-lithium (94 ml, 150 mmol) in dry THF (90 ml) 2-methylbut-1-yne (21 ml) was added through a cannula at −78 °C under an argon atmosphere. This mixture was stirred at room temperature for 30 min and then cooled to −78 °C. To this reagent solution a solution of the 22-aldehyde (3) (16 g, 37.7 mmol) in dry THF (30 ml) was added at −78 °C. This mixture was stirred at −78 °C for 1.5 h. The excess reagent was carefully destroyed with saturated aqueous ammonium chloride at −78 °C. Work-up (ethyl acetate for extraction) gave a crude product (17 g) which upon recrystallization from methanol provided the (22*R*)-22-ol (5) (7.0 g, 38.5%), m.p. 154—155 °C, $[\alpha]_D$ −21.2° (*c* 1.59, $CHCl_3$), δ_H (100 MHz) 0.70 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.18 (6 H, d, *J* 6.5 Hz, 26-H_3 and 27-H_3), 3.52 (2 H, m, 3-H and THP), 3.90 (1 H, m, THP), 4.46 (1 H, m, 22-H), 4.72 (1 H, m, THP), and 5.34 (1 H, m, 6-H) [Found: M^+, 380.3097. Calc. for $C_{27}H_{40}O$ (M^+ − THPOH): 380.3081].

The mother-liquor was again submitted to recrystallization from methanol to provide the (22*S*)-22-ol (4) (6.3 g, 35%), m.p. 129—130 °C, $[\alpha]_D$ −44.6° (*c* 0.84, $CHCl_3$), δ_H (100 MHz) 0.71 (3 H, s, 18-H_3), 1.01 (3 H, s, 19-H_3), 1.04 (3 H, d, *J* 6.5 Hz, 21-H_3), 1.16 (6 H, d, *J* 6.5 Hz, 26-H_3 and 27-H_3), 3.50 (2 H, m, 3-H and THP), 3.90 (1 H, m, THP), 4.41 (1 H, m, 22-H), 4.71 (1 H, m, THP), and 5.34 (1 H, m, 6-H) [Found: M^+, 380.3066. Calc. for $C_{27}H_{40}O$ (M^+ − THPOH): 380.3081].

To confirm the purity of the 22-stereoisomers (4) and (5), g.l.c. analysis was carried out as the corresponding 3,22-diols. G.c.l. conditions; a Shimadzu GC-7A, column; 1.5% OV-17 (2 m × 3.5 mm), flow rate; 50 ml/min, column temperature; 280 °C. Retention times were 5.4 min for the 22*S*-isomer and 6.1 min for the 22*R*-isomer.

(22S)-3β,22-*Dihydroxycholest*-5-*ene* (6a) *and* (22S)-3β,22-*Diacetoxycholest*-5-*ene* (6b).—The acetylenic alcohol (5) (100 mg) dissolved in ethyl acetate (5 ml) was stirred with 10 mg of 5% Pd-C in a hydrogen atmosphere at room temperature for 3 h. The catalyst was filtered off and the solvent removed under reduced pressure to leave a residue. This in methanol–THF (1 : 1, 5 ml) was treated with 3 drops of 2M-HCl at room temperature for 1 h. Work-up (ether for extraction) and recrystallization from methanol provided the diol (6a) (35 mg) m.p. 178—180 °C (lit.,[35] m.p. 180 °C);[30] δ_H (100 MHz) 0.70 (3 H, s, 18-H_3), 0.89 (9 H, d, *J* 6.5 Hz, 21-H_3, 26-H_3, and 27-H_3), 1.01 (3 H, s, 19-H_3), 3.30—3.75 (2 H, m, 22-H and 3-H), and 5.35 (1 H, m, 6-H).

This diol (6a) (30 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature for 18 h. Work-up (ether for extraction) and chromatography on silica gel (5 g) with benzene as eluant provided the diacetate (6b) (33 mg), which was recrystallized from methanol; it had m.p. 142—144.5 °C (lit.,[35] m.p. 146 °C),[30] δ_H (100 MHz) 0.69 (3 H, s, 18-H_3), 0.87 (6 H, d, *J* 6.5 Hz, 26-H_3 and 27-H_3), 1.01 (3 H, s, 19-H_3), 2.02 (6 H, s, two acetyls), 4.59 (1 H, m, 3-H), 4.94 (1 H, t, *J* 6 Hz, 22-H), and 5.35 (1 H, m, 6-H).

[22S,23(24)Z]-3β-*Tetrahydropyranyloxycholest*-5,23-*dien*-22-*ol* (7).—The mixture of (22*R*)-22-ol (5) (3.4 g, 7.05 mmol) and Lindlar catalyst (100 mg) in ether–ethanol (1 : 1; 260 ml) was stirred at room temperature under a hydrogen atmosphere for 3 h. Filtration and removal of the solvent under reduced pressure provided the allylic alcohol (7) (3.4 g), m.p. 156—158 °C (methanol), $[\alpha]_D$ −42.9° (*c* 0.468, $CHCl_3$); δ_H (100 MHz) 0.68 (3 H, s, 18-H_3), 0.95 (3 H, d, *J* 6.5 Hz, 21-H_3), 0.97 (6 H, d, *J* 6.5 Hz, 26-H_3 and 27-H_3), 1.01 (3 H, s, 19-H_3), 3.50 (2 H, m, 3-H and THP), 3.90 (1 H, m, THP), 4.54 (1 H, d, *J* 8 Hz, 22-H), 4.69 (1 H, m, THP), and 5.32 (3 H, m, 6-H, 23-H, and 24-H) (Found: *m/z* 382.3216. Calc. for $C_{27}H_{42}O$: M^+ − THPOH, 382.3238).

(22R,23R,24R)-23,24-*Epoxy*-3β-*tetrahydropyranyloxycholest*-5-*en*-22-*ol* (8).—A suspension of 70% t-butyl hydroperoxide (3 ml) in dry benzene (5 ml) was added to a solution of the allylic alcohol (7) (2.39 g, 4.94 mmol) in dry benzene (50 ml) at room temperature under an argon atmosphere. To this mixture vanadyloxy acetylacetonate (80 mg) was added. This mixture was stirred at room temperature for 2.5 h. To this reaction mixture saturated aqueous sodium carbonate was added. Work-up (ethyl acetate for extraction) gave a crude product (2.5 g), chromatography of which on silica gel (50 g) with benzene–ethyl acetate (25 : 1) as eluant provided the epoxy alcohol (8) (2.10 g, 85%), m.p. 190—191 °C (methanol), δ_H (100 MHz) 0.69 (3 H s, 18-H_3), 0.97 (3 H, d, *J* 6 Hz, 21-H_3), 1.00 (3 H, s, 19-H_3), 1.08 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 2.66 (1 H, dd, *J* 8 and 4 Hz, 24-H), 3.07 (1 H, dd, *J* 8 and 4 Hz, 23-H), 2.54 (3 H, m, 3-H, 22-H, and THP), 3.89 (1 H, m, THP), 4.70 (1 H, m, THP), and 5.33 (1 H, m, 6-H) (Found: *m/z* 398.3178. Calc. for $C_{27}H_{42}O_2$: M^+ − THPOH, 398.3187).

(22R,23R,24S)-24-*Cyanocholest*-5-*ene*-3β,22,23-*triol Triacetate* (9a) *and* (22R,23S,24R)-23-*Cyanocholest*-5-*ene*-3β,22,23-*triol Triacetate* (9b).—The epoxy alcohol (8) (20 mg, 0.048 mmol) in THF (4 ml) was treated with hydrogen cyanide–THF (0.65 ml, 4.4 mmol) and triethylaluminium/hexane (2 ml, 4.5 mmol) at 0 °C for 16 h. To this reaction mixture 10% aqueous potassium hydroxide was added. Work-up (ethyl acetate for extraction) gave a crude product (20 mg), which in methanol–THF (1 : 1; 3 ml) was treated with 3 drops of 2M-HCl at room temperature for 1 h. Work-up (ethyl acetate for extraction) and subsequent acetylation provided a mixture of the triacetate (9a) and (9b) (18 mg). These were separated by preparative thin-layer chromatography [benzene–ethyl acetate (4 : 1), developed twice] to provide the less polar 23-cyano compound (9b) (5.8 mg), $\nu_{max.}$ ($CHCl_3$) 1 745s, 1 380s, 1 250s, and 1 030s cm^{-1}; δ_H (100 MHz) 0.74 (3 H, s, 18-H_3), 1.02 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 1.03 (3 H, s, 19-H_3), 2.05 (3 H, s, acetyl), 2.07 (3 H, s, acetyl), 2.11 (3 H, s, acetyl), 3.18 (1 H, dd, *J* 10 and 3 Hz, 23-H), 4.58 (1 H, m, 3-H), 4.70 (1 H, dd, *J* 10 and 3 Hz, 24-H), 5.18 (1 H, d *J* 10 Hz, 22-H), and 5.34 (1 H, m, 6-H); and the more polar 24-cyano compound (9a) (9.3 mg), $\nu_{max.}$ ($CHCl_3$) 1 740s, 1 372s, 1 240s, and 1 020s cm^{-1}; δ_H (100 MHz) 0.74 (3 H, s, 18-H_3), 1.03 (3 H,

s, 19-H_3), 2.04 (3 H, s, acetyl), 2.06 (3 H, s, acetyl), 2.10 (3 H, s, acetyl), 4.57 (1 H, m, 3-H), 5.28 (2 H, bs, 22-H and 23-H), and 5.34 (1 H, m, 6-H).

(22R,23R,24R)-23,24-*Epoxy*-3β-*tetrahydropyranyloxy*-*cholest*-5-*en*-22-*ol* 22-*Acetate* (10).—A mixture of the epoxy alcohol (8) (2.56 g, 5.11 mmol) and acetic anhydride (5 ml) in pyridine (10 ml) was stirred at room temperature for 16 h. To this reaction mixture ice was added. Work-up (ethyl acetate for extraction) and chromatography on silica gel (50 g) with benzene–ethyl acetate (50 : 1) as eluant provided the epoxy acetate (10) (2.71 g, 98%), m.p. 149—150 °C (methanol), $[\alpha]_D$ −66.9° (*c* 1.36, $CHCl_3$), δ_H (100 MHz) 0.70 (3 H, s, 18-H_3), 1.00 (3 H, d, *J* 6 Hz, 21-H_3), 1.01 (3 H, s, 19-H_3), 1.12 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 2.10 (3 H, s, acetyl), 2.64 (1 H, dd, *J* 10 and 4 Hz, 24-H), 3.17 (1 H, dd, *J* 8 and 4 Hz, 23-H), 3.54 (2 H, m, 3-H and THP), 3.90 (1 H, m, THP), 4.71 (1 H, m, THP), 4.95 (1 H, d, *J* 8 Hz, 22-H), and 5.34 (1 H, m, 6-H).

(22R,23R,24S)-24-*Cyanocholest*-5-*ene*-3β,22,23-*triol* 22,23-*Acetonide* (11).—A solution of triethylaluminium in hexane (20 ml, 45 mmol) was placed in flask under argon atmosphere. THF (50 ml) was added at room temperature. To this solution, a solution of hydrogen cyanide in THF (6.7 ml, 45.6 mmol) was added at 0 °C. After the mixture had been stirred for 15 min, a solution of the epoxy acetate (10) (1.82 g, 3.60 mmol) in THF (30 ml) was added at 0 °C. This mixture was stirred at 0 °C and allowed to reach room temperature under an argon atmosphere during 24 h. To this reaction mixture 10% aqueous potassium hydroxide was added. Work-up (ethyl acetate for extraction) gave a crude product (1.9 g). This was treated with 5% KOH–MeOH (15 ml) and ether (15 ml) at room temperature for 1 h. Work-up (ethyl acetate for extraction) gave a crude product (1.86 g). This in THF–methanol (1 : 1; 20 ml) was treated with 0.5 ml of 2M-HCl at room temperature for 1 h. Work-up (ethyl acetate for extraction) provided a crude product (1.7 g), which was dissolved in acetone (30 ml). Toluene-*p*-sulphonic acid (50 mg) was added to the mixture which was then stirred at room temperature for 17 h. Work-up (ethyl acetate for extraction) and chromatography on silica gel (40 g) with benzene–ethyl acetate (25 : 1) as eluant provided the acetonide (11) (985 mg, 56%), m.p. 222—224 °C (methanol; δ_H (100 MHz) 0.70 (3 H, s, 18-H_3), 1.01 (3 H, d, *J* 6 Hz, 21-H_3), 1.03 (3 H, s, 19-H_3), 1.10 (3 H, d, *J* 6 Hz, 26-H_3), 1.17 (3 H, d, *J* 6 Hz, 27-H_3), 1.40 (3 H, s, acetonide), 1.46 (3 H, s, acetonide), 3.53 (1 H, m, 3-H), 3.82 (1 H, dd, *J* 8 and 3 Hz, 22-H or 23-H), 4.06 (1 H, d, *J* 8 Hz, 22-H or 23-H), and 5.38 (1 H, m, 6-H); δ_C (25.1 MHz) 11.7, 12.7, 19.4, 20.4, 21.1 (2 × C), 24.1, 27.0, 27.2, 28.0, 29.3, 31.6, 31.8, 31.9, 35.6, 36.5, 37.2, 39.5, 42.3 (3 × C), 50.0, 53.1, 56.5, 71.6, 74.7, 81.1, 109.2, 118.4, 121.5, and 140.8 (Found: *m/z* 483.3694. Calc. for $C_{31}H_{49}NO_3$: *M*, 483.3714).

(22R,23R,24S)-24-*Formylcholest*-5-*ene*-3β,22,23-*triol* 22,23-*Acetonide* (12).—To a solution of the nitrile (11) (1.0 g, 2.1 mmol) in THF (10 ml) a solution of di-isobutylaluminium hydride in hexane (2.5 ml, 4.4 mmol) was added at room temperature. This mixture was stirred for 5 h. Work-up (ether for extraction) and chromatography on silica gel (20 g) with benzene–ethyl acetate (50 : 1) as eluant provided the aldehyde (12) (560 mg, 65%), m.p. 167—171 °C (methanol), $\nu_{max.}$ ($CHCl_3$) 1 718 cm^{-1}; δ_H (100 MHz) 0.70 (3 H, s, 18-H_3), 1.01 (3 H, s, 19-H_3), 1.31 (3 H, s, acetonide), 1.37 (3 H, s, acetonide), 3.48 (1 H, m, 3-H), 3.69 (1 H, d, *J* 9 Hz, 22-H or 23-H), 4.06 (1 H, dd, *J* 9 and 3 Hz, 22-H or 23-H), 5.34 (1 H, d, 6-H), and 9.72 (1 H, d, *J* 4 Hz, 28-H).

(22R,23R,24S)-3β-*Acetyloxyergost*-5-*ene*-22,23,28-*triol* 22,23-*Acetonide* (13a).—A mixture of the aldehyde (12) (500 mg, 1.03 mmol) and acetic anhydride (3 ml) in pyridine (5 ml) was stirred at room temperature for 16 h. Work-up (ethyl acetate for extraction) gave a crude product (580 mg) which was dissolved in methanol–THF (1 : 1, 20 ml) and treated with sodium borohydride (200 mg, 5.25 mmol) at room temperature for 3 h. Work-up (ether for extraction) and chromatography on silica gel (20 g) with benzene–ethyl acetate (50 : 1) as eluant provided the 28-alcohol (13a) (525 mg), m.p. 168—170 °C (methanol), $[\alpha]_D$ −22.9° (*c* 1.24, $CHCl_3$), δ (100 MHz) 0.68 (3 H, s, 18-H_3), 0.92 (3 H, d, *J* 6 Hz, 21-H_3), 1.02 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 1.03 (3 H, s, 19-H_3), 1.37 (3 H, s, acetonide), 1.39 (3 H, s, acetonide), 2.02 (3 H, s, acetyl), 3.92 (1 H, t, *J* 6 Hz, hydroxy), 3.78 (2 H, m, 22-H and 23-H), 3.96 (2 H, bs, 28-H_2), 4.58 (1 H, m, 3-H), 5.35 (7 H, m, 6-H) (Found: *m/z* 470.3764. Calc. for $C_{31}H_{50}O_3$: M^+ − AcOH, 470.3763).

(22R,23R,24S)-3β-*Acetyloxy*-28-*iodoergost*-5-*ene*-22,23-*diol* 22,23-*Acetonide* (13c).—The 28-alcohol (13a) (233 mg, 0.434 mmol) in pyridine (5 ml) was treated with methanesulphonyl chloride (0.1 ml) at room temperature for 3 h. Work-up (ether for extraction) provided the crude mesylate (13b) (260 mg); δ_H (100 MHz) 0.69 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.35 (6 H, s, acetonide), 2.02 (3 H, s, acetyl), 3.01 (3 H, s, mesyl), 3.87 (2 H, br s, 28-H_2), 4.36 (2 H, m, 22-H and 23-H), 4.50 (1 H, m, 3-H), and 5.35 (1 H, m, 6-H).

This mesylate (13b) (260 mg) was dissolved in acetone (20 ml) and sodium iodide (300 mg) was added to it; the mixture was then refluxed for 72 h. Work-up (ethyl acetate for extraction) and chromatography on silica gel (15 g) with benzene as eluant provided the iodide (13c) (258 mg, 96%), m.p. 131—133 °C (methanol), δ_H (100 MHz) 0.71 (3 H, s, 18-H_3), 0.93 (3 H, d, *J* 6 Hz, 21-H_3), 0.97 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 1.03 (3 H, s, 19-H_3), 1.35 (6 H, s, acetonide), 2.03 (3 H, s, acetyl), 3.24 (2 H, m, 28-H_2), 3.82 (1 H, d, *J* 8 Hz, 22-H or 23-H), 3.94 (1 H, d, *J* 8 Hz, 22-H or 23-H), 4.56 (1 H, m, 3-H), and 5.35 (1 H, m, 6-H) (Found: *m/z* 580.2773. Calc. for $C_{31}H_{49}IO_2$: M^+ − AcOH 580.2780).

(22R,23R,24S)-3β-*Acetyloxyergost*-5-*ene*-22,23-*diol* 22,23-*Acetonide* (13d).—The iodide (13c) (258 mg, 0.403 mmol) in THF (10 ml) was treated with tributyltin hydride (1 ml) under an argon atmosphere at room temperature for 18 h. Removal of the solvent under reduced pressure and chromatography on silica gel (10 g) with benzene as eluant provided the acetate (13d) (203 mg, 98%), m.p. 153—155 °C (methanol), $[\alpha]_D$ −21.9° (*c* 1.26, $CHCl_3$), δ_H (100 MHz) 0.69 (3 H, s, 18-H_3), 0.85 (3 H, d, *J* 6 Hz, 21-H_3 or 28-H_3), 0.89 (3 H, d, *J* 6 Hz, 21-H_3 or 28-H_3), 0.93 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 1.03 (3 H, s, 19-H_3), 1.35 (3 H, s, acetonide), 1.36 (3 H, s, acetonide), 2.02 (3 H, s, acetyl), 3.70 (1 H, dd, *J* 8 and 4 Hz, 22-H or 23-H), 3.94 (1 H, d, *J* 8 Hz, 22-H or 23-H), 4.56 (1 H, m, 3-H), and 5.36 (1 H, m, 6-H) (Found: *m/z*, 454.3806. Calc. for $C_{31}H_{50}O_2$: M^+ − AcOH, 454.3814).

(22R,23R,24S)-3β,22,23-*Triacetoxyergost*-5-*ene* (14a).—The acetate (13d) (20 mg, 0.0389 mmol) was treated with acetic acid (5 ml) and water (1 ml) under reflux for 6 h. Removal of the solvent under reduced pressure gave the residue, which was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) at 60 °C for 15 h. Work-up (ethyl acetate for extraction) gave a crude product (34 mg), which was applied to a column of silica gel (5 g). Elution with benzene–ethyl acetate (50 : 1) provided the triacetate (14a) (21 mg, 97%), m.p. 140—141 °C (methanol), δ_H (100 MHz) 0.72 (3 H, s, 18-H_3), 1.98 (3 H, s, acetyl), 2.04 (6 H, s, two acetyls), 4.54 (1 H, m, 3-H), 5.12

(1 H, d, *J* 9 Hz, 22-H), 5.30 (1 H, d, *J* 9 Hz, 23-H), and 5.34 (1 H, m, 6-H).

(22R,23R,24S)-3β,22,23-*Trihydroxyergost*-5-*ene* (14b).—The triacetate (14a) (20 mg, 0.0382 mmol) was treated with 5% KOH–MeOH (7 ml) under reflux for 1 h. Work-up (ethyl acetate for extraction) provided the triol (14b) (15 mg, 98%), m.p. 205—207 °C (methanol), δ (200 MHz, [$^2H_5$]pyridine) 0.81 (3 H, s, 18-H_3), 1.05 (3 H, s, 19-H_3), 3.85 (1 H, m, 3-H), 3.99 (1 H, d, *J* 8 Hz, 22-H), 4.13 (1 H, d, *J* 8 Hz, 23-H), and 5.43 (1 H, m, 6-H); EI-MS, *m*/*z* 432 (M^+), 414, 399, 381, 361 ($M^+ - 71$, C_{23}–C_{24} fission), 343, 332 ($M^+ - 100$, C_{22}–C_{23} fission, accompanied by H-transfer), 313 (base peak), 295, 273 ($M^+ - 159$, C_{17}–C_{20} fission), 255, 159, 131, 101, 71, and 43.

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-3β-*methylsulphonyloxyergost*-5-*ene* (13e).—A mixture of the acetate (13d) (440 mg, 0.855 mmol) and 5% KOH–MeOH (5 ml) in THF (7 ml) was stirred at room temperature for 1 h. Work-up (ether for extraction) gave a crude product (396 mg) which, in pyridine (3 ml), was treated with methanesulphonyl chloride (0.1 ml) at room temperature for 2 h. Work-up (ether for extraction) provided the mesylate (13e) (463 mg), δ_H (100 MHz) 0.69 (3 H, s, 18-H_3), 0.85 (3 H, d, *J* 6 Hz, 21-H_3 or 28-H_3), 0.89 (3 H, d, *J* 6 Hz, 21-H_3 or 28-H_3), 0.93 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 1.03 (3 H, s, 19-H_3), 1.35 (3 H, s, acetonide), 1.36 (3 H, s, acetonide), 3.00 (3 H, s, mesyl), 3.70 (1 H, dd, *J* 8 and 4 Hz, 22-H or 23-H), 3.94 (1 H, d, *J* 8 Hz, 22-H or 23-H), 4.50 (1 H, d, 3-H), and 5.34 (1 H, m, 6-H).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-3β-*methylsulphonyloxy*-5α-*ergostan*-6-*one* (15).—The mesylate (13e) (463 mg) in THF (7 ml) was treated with BH_3–THF complex (3 ml, 3.0 mmol) under an argon atmosphere at room temperature for 3.5 h. Excess of reagent was destroyed with water after which 2M-NaOH (1 ml) and 30% H_2O_2 (1 ml) were added to the reaction mixture. It was then stirred at room temperature for 30 min. Work-up (ether for extraction) provided a crude product (443 mg) which was dissolved in dichloromethane (20 ml); pyridinium chlorochromate (700 mg, 3.25 mmol) was then added to the solution and the whole stirred at room temperature for 3.5 h. The mixture was diluted with ether (100 ml) and filtered through a short column of Florisil; removal of solvent under reduced pressure provided the 6-oxo steroid (15) (389 mg); δ_H (100 MHz) 0.66 (3 H, s, 18-H_3), 0.78 (3 H, s, 19-H_3), 1.35 (3 H, s, acetonide), 1.36 (3 H, s, acetonide), 3.00 (3 H, s, mesyl), 3.70 (1 H, dd, *J* 8 and 4 Hz, 22-H or 23-H), 3.94 (1 H, d, *J* 8 Hz, 22-H or 23-H), and 4.60 (1 H, m, 3-H).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-5α-*ergost*-2-*en*-6-*one* (16).—A mixture of the mesylate (15) (389 mg) and lithium bromide (300 mg, 3.45 mmol) in dimethylformamide (7 ml) was stirred under reflux for 1 h. Work-up (ethyl acetate for extraction) gave a crude product (335 mg), which was applied to a column of silica gel (20 g). Elution with benzene–hexane (5 : 1) provided the 2-ene (16) [209 mg, 60% from (13d)], m.p. 235—237 °C (methanol), $\nu_{max.}$ ($CHCl_3$) 1 710 cm^{-1}; δ_H (100 MHz) 0.70 (3 H, s, 18-H_3), 0.73 (3 H, s, 19-H_3), 1.35 (3 H, s, acetonide), 1.36 (3 H, s, acetonide), 3.70 (1 H, dd, *J* 8 and 4 Hz, 22-H or 23-H), 3.94 (1 H, d, *J* 8 Hz, 22-H or 23-H), and 5.50 (2 H, m, 2-H and 3-H) (Found: *m*/*z* 470.3387. Calc. for $C_{30}H_{46}O_4$: *M*, 470.3398).

(22R,23R,24S)-2α,3α,22,23-*Tetrahydroxy*-5α-*ergostan*-6-*one* (2a).—To a solution of the 2-ene (16) (200 mg, 0.426 mmol) in t-butyl alcohol–THF–water (10 : 10 : 1; 15 ml) *N*-methylmorpholine *N*-oxide (150 mg, 1.28 mmol) and osmium tetraoxide (20 mg) were added. This mixture was stirred at room temperature for 3 h after which time water (5 ml) and sodium hydrogen sulphide (200 mg) were added to it. This mixture was stirred at room temperature for 1 h. Work-up (ethyl acetate for extraction) provided a crude product (220 mg) which was treated with 70% aqueous acetic acid at 65 °C for 4 h. Removal of the solvent under reduced pressure provided the crude castasterone (2a) (219 mg), which was recrystallized from ethyl acetate; it had m.p. 250—252 °C, δ_H (400.5 MHz, [$^2H_5$]pyridine) 0.74 (3 H, s, 18-H_3), 0.85 (3 H, s, 19-H_3), 1.04 (3 H, d, *J* 7.1 Hz, 28-H_3), 1.11 (3 H, d, *J* 7.1 Hz, 26-H_3), 1.15 (3 H, d, *J* 7.1 Hz, 27-H_3), 1.23 (3 H, d, *J* 7.1 Hz, 21-H_3), 2.32 (1 H, dt, *J* 15.7 and 4.3 Hz), 2.36 (1 H, dd, *J* 12.9 and 4.3 Hz), 3.13 (1 H, dd, *J* 12.9 and 2.9 Hz, 5-H), 3.98 (1 H, d, *J* 8.6 Hz, 22-H), 4.06 (1 H, m, $W_{\frac{1}{2}}$ 20 Hz, 2-H), 4.15 (1 H, d, *J* 8.6 Hz, 23-H), 4.43 (1 H, m, $W_{\frac{1}{2}}$ 8.6 Hz, 3-H); EI-MS, *m*/*z* 446 ($M^+ - 18$), 394, 393 ($M^+ - 71$, C_{23}–C_{24} fission), 364 ($M^+ - 101$, C_{22}–C_{23} fission accompanied by H-transfer, base peak), 363, 345, 327, 287, 263, 245, 175, 173, 155, 147, 107, 101, 95, and 43. Emitter CI-MS (isobutane), *m*/*z* 465 ($M^+ + 1$, base peak), 447, and 429.

(22R,23R,24S)-2α,3α,22,23-*Tetra-acetoxy*-5α-*ergostan*-6-*one* (17).—A mixture of the crude castasterone (2a) (204 mg) and acetic anhydride (3 ml) in pyridine (6 ml) in the presence of 4-dimethylaminopyridine (20 mg) was stirred at 60 °C for 17 h. Work-up (ethyl acetate for extraction) and chromatography on silica gel (20 g) with benzene–ethyl acetate (5 : 1) as eluant provided the tetra-acetate (17) [215 mg, 80% from (16), m.p. 215—217 °C (methanol), $[\alpha]_D$ −6.82° (*c* 1.28, $CHCl_3$), δ_H (100 MHz) 0.70 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 0.96 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 2.02 (6 H, s, two acetyls), 2.04 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 2.54 (1 H, dd, *J* 10 and 5 Hz, 5-H), 4.92 (1 H, m, 2-H), and 5.04—5.42 (3 H, m, 3-H, 22-H, and 23-H).

(22R,23R,24S)-2α,3α,22,23-*Tetra-acetoxy*-24-*methyl*-B-*homo*-7-*oxa*-5α-*cholestan*-6-*one* (18).—To a solution of 90% H_2O_2 (0.625 ml) in dichloromethane (15 ml) trifluoroacetic anhydride (4 ml) was added at 0 °C. This mixture was stirred at 0 °C for 5 min. To a mixture of the 6-oxosteroid (17) (150 mg, 0.237 mmol) and disodium hydrogen phosphate (630 mg) in dichloromethane (6.3 ml) the above-prepared trifluoroperacetic acid solution (4.3 ml) was added at 0 °C. This mixture was stirred at 0 °C for 3 h after which saturated aqueous sodium hydrogen sulphite was added. Work-up (ethyl acetate for extraction) and chromatography on silica gel (30 g) with benzene–ethyl acetate (10 : 1) as eluant provided the brassinolide tetra-acetate (18) (130 mg, 85%), m.p. 231—233 °C (methanol), $[\alpha]_D$ +21.6° (*c* 1.04, $CHCl_3$), δ_H (100 MHz) 0.74 (3 H, s, 18-H_3), 0.98 (3 H, s, 19-H_3), 1.99 (6 H, s, two acetyls), 2.01 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 3.00 (1 H, dd, *J* 13 and 5 Hz, 5-H), 4.06 (2 H, m, 7-H_2), 4.86 (1 H, m, 2-H), and 5.00—5.40 (3 H, m, 3-H, 22-H and 23-H).

(22R,23R,24S)-2α,3α,22,23-*Tetrahydroxy*-24-*methyl*-B-*homo*-7-*oxa*-5α-*cholestan*-6-*one* (*Brassinolide*) (1a).—The tetra-acetate (18) (130 mg, 0.20 mmol) was treated with 5% KOH/MeOH (10 ml) under reflux for 1 h. The mixture was cooled to room temperature and 6M-HCl (10 ml) added to it; it was then stirred at room temperature for 1 h. Work-up (ethyl acetate for extraction) provided brassinolide (1a) (80 mg, 84%), which was recrystallized from aqueous methanol; m.p. 273—278 °C (lit.,[1] m.p. 274—275 °C), $[\alpha]_D$ +16.0° (*c* 0.985, CH_2Cl_2–MeOH, 1 : 1), $\nu_{max.}$ (KBr) 3 450s, 2 975s, 2 945s, 2 870m, 2 850m, 1 730m, 1 700s, 1 690sh, 1 640w, 1 463m, 1 443m, 1 410m, 1 388m, 1 335m, 1 320m, 1 300w, 1 285m, 1 260m, 1 230m, 1 190m, 1 170w, 1 148m, 1 130m, 1 120m, 1 097m, 1 070s, 1 040m, 1 030m, 990m, and 970w;

δ_H (400.5 MHz, [$^2H_5$]pyridine) 0.72 (3 H, s, 18-H_3), 1.04 (3 H, d, *J* 7.1 Hz, 28-H_3), 1.06 (3 H, s, 19-H_3), 1.11 (3 H, d, *J* 7.1 Hz, 26-H_3), 1.15 (3 H, d, *J* 7.1 Hz, 27-H_3), 1.21 (3 H, d, *J* 7.1 Hz, 21-H_3), 2.32 (1 H, dt, *J* 15.7 and 4.3 Hz), 2.52 (1 H, t, *J* 12.9 Hz), 3.60 (1 H, dd, *J* 12.9 and 4.3 Hz, 5-H), 3.95 (1 H, d, *J* 8.6 Hz, 22-H), 4.00—4.10 (3 H, m, 7-H_2 and 2-H), 4.13 (1 H, d, *J* 8.6 Hz, 23-H), 4.44 (1 H, bs, $W_{\frac{1}{2}}$ 8.6 Hz, 3-H); δ_C (50 MHz, CD_2Cl_2–CD_3OD, 9 : 1) δ 10.3, 11.8, 12.1, 15.6, 20.8, 21.0, 22.6, 25.1, 27.9, 29.4, 31.3, 31.7, 37.3, 38.6, 39.6, 40.1, 40.5, 41.4, 41.6, 42.8, 47.6, 58.7, 68.3, 68.4, 71.0, 73.5, 74.7, and 177.6; EI-MS, *m/z* 480 (M^+), 465, 462, 447, 409, 380 (base peak, C_{22} – C_{23} fission accompanied by H-transfer), 379, 361, 350, 343, 331, 325, 322, 313, 307, 303, 285, 177, 173, 155, 131, 101, 71, 43; Emitter CI-MS (isobutane), *m/z* 481 (M^+ + 1, base peak), 463, 445 (Found: *m/z* 380.2564. Calc. for $C_{22}H_{36}O_5$: M^+ – 100, 380.2562).

(22R,23R)-3β,22,23-*Trihydroxycholest*-5-*ene* (19a).—The epoxy alcohol (8) (700 mg, 1.40 mmol) in dioxane (15 ml) was treated with chloromethyl methyl ether (5 ml) and diethylcyclohexylamine (7 ml) at room temperature for 16 h. Work-up (ethyl acetate for extraction) provided the crude product (760 mg). This in THF (10 ml) was treated with lithium aluminium hydride (500 mg) under reflux for 15 h. Work-up and acetylation provided the 23-acetate (786 mg), δ_H ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 1.01 (3 H, s, 19-H_3), 2.05 (3 H, s, acetyl), 3.20—4.10 (3 H, m, THP and 3-H), 3.35 (3 H, s, methoxymethyl), 4.60—4.80 (3 H, m, methoxymethyl and THP), 5.00 (1 H, m, 23-H), and 5.32 (1 H, m, 6-H). This acetate in THF (15 ml) was treated with 30% $HClO_4$ (3 ml) and 60 °C for 5 h. Work-up (ethyl acetate for extraction) gave a crude product, which was saponified with 5% KOH–MeOH (15 ml) under reflux for 1 h. Work-up (dichloromethane for extraction) and chromatography on silica gel (50 g) using benzene–ethyl acetate (20 : 1) as eluant provided the (22*R*,23*R*)-triol (19a) (409 mg, 70%), m.p. 187—191 °C (methanol), δ_H (100 MHz) 0.72 (3 H, s, 18-H_3), 0.94 (3 H, d, *J* 7 Hz, 21-H_3), 0.96 (6 H, d, *J* 7 Hz, 26-H_3 and 27-H_3), 1.03 (3 H, s, 19-H_3), 3.28—3.78 (3 H, m, 3-H, 22-H, and 23-H), and 5.35 (1 H, m, 6-H) (Found: *m/z* 418.3428. Calc. for $C_{27}H_{46}O_3$: M^+, 418.3449).

(22R,23R)-3β,22,23-*Triacetoxycholest*-5-*ene* (19b).—The (22*R*,23*R*)-triol (19a) (20 mg, 0.0478 mmol) was treated with acetic anhydride (1 ml) and pyridine (1 ml) at 60 °C for 18 h. Work-up (ethyl acetate for extraction) gave a crude product (23 mg), which was applied to a column of silica gel (5 g). Elution with benzene–ethyl acetate (50 : 1) provide the triacetate (19b) (21 mg), m.p. 159—160 °C (methanol), $[\alpha]_D$ −5.2° (*c* 0.988, $CHCl_3$), δ_H (100 MHz) 0.69 (3 H, s, 18-H_3), 0.91 (6 H, d, *J* 6 Hz, 26-H_3 and 27-H_3), 1.01 (3 H, s, 19-H_3), 1.98 (3 H, s, acetyl), 2.02 (6 H, s, two acetyls), 4.56 (1 H, m, 3-H), 4.96 (1 H, d, *J* 8 Hz, 22-H), 5.14 (1 H, ddd, *J* 8, 2.5, and 2 Hz, 23-H), and 5.32 (1 H, m, 6-H).

(22R,23R)-3β-*Methylsulphonyloxy*-22,23-*dihydroxycholest*-5-*ene* 22,23-*Acetonide* (20).—The (22*R*,23*R*)-triol (19a) (465 mg, 1.11 mmol) in acetone (20 ml) was treated with toluene-*p*-sulphonic acid (5 mg) at room temperature for 17 h. Work-up (ether for extraction) gave a crude product (508 mg) which was treated with methanesulphonyl chloride (0.4 ml, 5.17 mmol) and pyridine (3 ml) at room temperature for 15 h. Work-up (ether for extraction) gave the mesylate (20) (590 mg, 98%), δ_H (100 MHz) 0.70 (3 H, s, 18-H_3), 0.95 (3 H, d, *J* 6.5 Hz, 21-H_3), 0.98 (6 H, d, *J* 7 Hz, 26-H_3 and 27-H_3), 1.03 (3 H, s, 19-H_3), 1.36 (6 H, s, acetonide), 3.00 (3 H, s, mesyl), 3.61 (1 H, d, *J* 8 Hz, 22-H or 23-H), 3.78 (1 H, dt, *J* 8 and 4 Hz, 22-H or 23-H), 4.50 (1 H, m, 3-H), and 5.41 (1 H, m, 6-H).

(22R,23R)-3β-*Methylsulphonyloxy*-22,23-*dihydroxy*-5α-*cholestan*-6-*one* 22,23-*Acetonide* (21).—The mesylate (20) (590 mg, 1.10 mmol) was converted, as described for (15), into the 6-oxo steroid (21) (278 mg, 50%), amorphous, $\nu_{max.}$ ($CHCl_3$) 1 711 cm^{-1}; δ_H (100 MHz) 0.68 (3 H, s, 18-H_3), 0.78 (3 H, s, 19-H_3), 0.90 (3 H, d, *J* 6.5 Hz, 21-H_3), 1.38 (6 H, s, acetonide), 3.02 (3 H, s, mesyl), 3.60 (1 H, d, *J* 8 Hz, 22-H or 23-H), 3.78 (1 H, dt, *J* 8 and 4 Hz, 22-H or 23-H), and 4.60 (1 H, m, 3-H).

(22R,23R)-22,23-*Dihydroxy*-5α-*cholest*-2-*en*-6-*one* 22,23-*Acetonide* (22).—The mesylate (21) (278 mg, 0.503 mmol) was treated as described for (16) to provide the 2-ene (22) (195 mg, 85%), m.p. 186—188 °C (methanol), $\nu_{max.}$ ($CHCl_3$) 1 710 cm^{-1}; δ_H (100 MHz) 0.69 (3 H, s, 18-H_3), 0.72 (3 H, s, 19-H_3), 1.37 (6 H, s, acetonide), 3.61 (1 H, d, *J* 8 Hz, 22-H or 23-H), 3.78 (1 H, dt, *J* 8 and 4 Hz, 22-H or 23-H), 5.60 (2 H, m, 2-H and 3-H) (Found: *m/z* 456.3606. Calc. for $C_{30}H_{48}O_3$: M^+, 456.3605).

(22R,23R)-2α,3α,22,23-*Tetrahydroxy*-5α-*cholestan*-6-*one* (2b).—The 2-ene (22) (105 mg, 0.230 mmol) was converted, as described for (2a), into the tetrahydroxy-6-oxo steroid (2b) (102 mg, crude). Recrystallization from ethyl acetate (twice) provided (2b) (24 mg), m.p. 254—255 °C, δ_H (400.5 MHz, [$^2H_5$]pyridine) 0.70 (3 H, s, 1-8H_3), 0.85 (3 H, s, 19-H_3), 1.03 (3 H, d, *J* 7.1 Hz, 26-H_3), 1.06 (3 H, d, *J* 7.1 Hz, 27-H_3), 1.24 (3 H, d, *J* 7.1 Hz, 21-H_3), 2.32 (1 H, dt, *J* 15.7 and 4.3 Hz), 2.36 (1 H, dd, *J* 12.9 and 4.3 Hz), 3.13 (1 H dd, *J* 12.9 and 2.9 Hz, 5-H), 3.77 (1 H, dd, *J* 8.0 and 1.4 Hz, 22-H), 4.01—4.10 (2 H, m, 2-H and 23-H), and 4.43 (1 H, m, $W_{\frac{1}{2}}$ 8.6 Hz, 3-H); EI-MS, *m/z* 364 (M^+ – 87, C_{22}–C_{23} fission, accompanied by H-transfer), 346 (base peak), 334, 327, 316, 287, 269, 87, and 43 (Found: *m/z* 364.2636. Calc. for $C_{22}H_{36}O_4$: M^+ – 87, 346.2615).

(22R,23R)-2α,3α,22,23-*Tetra-acetoxy*-5α-*cholestan*-6-*one* (23).—The tetraol (2b) (141 mg) was acetylated, as described for (17), to provide the tetra-acetate (23) (190 mg, 97%), δ_H (100 MHz) 0.65 (3 H, s, 18-H_3), 0.79 (3 H, s, 19-H_3), 0.86 (6 H, d, *J* 6.5 Hz, 26-H_3 and 27-H_3), 1.93 (6 H, s, two acetyls), 1.97 (3 H, s, acetyl), 2.02 (3 H, s, acetyl), and 4.60—5.30 (4 H, m, 2-H, 3-H, 22-H and 23-H).

(22R,23R)-2α,3α,22,23-*Tetra-acetoxy*-B-*homo*-7-*oxa*-5α-*cholestan*-6-*one* (24).—The tetra-acetoxy-6-oxo-steroid (23) (190 mg, 0.305 mmol) was submitted to Baeyer-Villiger oxidation as described for (18), to give the 7-*oxalactone* (24) (160 mg, 82%) as an oil, δ_H (100 MHz) 0.69 (3 H, s, 18-H_3), 0.93 (3 H, s, 19-H_3), 1.94 (6 H, s, two acetyls), 1.96 (3 H, s, acetyl) 2.04 (3 H, s, acetyl), 3.00 (1 H, dd, *J* 13 and 6 Hz, 5-H), 4.05 (2 H, m, 7-H_2), and 4.50—5.40 (4 H, m, 2-H, 3-H, 22-H, and 23-H).

(22R,23R)-2α,3α,22,23-*Tetrahydroxy*-B-*homo*-7-*oxa*-5α-*cholestan*-6-*one* (1b).—The tetra-acetate (24) (150 mg, 0.235 mmol) was deprotected as described for (18), to provide (22*R*,23*R*)-28-norbrassinolide (1b) (96 mg, 88%), m.p. 256—259 °C (aqueous methanol), $[\alpha]_D$ +32.0° (*c* 1.15, MeOH), δ_H (400.5 MHz, [$^2H_5$]pyridine) 0.69 (3 H, s, 18-H_3), 1.03 (3 H, d, *J* 7.1 Hz, 26-H_3), 1.05 (3 H, s, 19-H_3), 1.06 (3 H, d, *J* 7.1 Hz, 27-H_3), 1.21 (3 H, d, *J* 7.1 Hz, 21-H_3), 2.31 (1 H, dt, *J* 14.3 and 4.3 Hz), 2.51 (1 H, t, *J* 14.3 Hz), 3.60 (1 H, dd, *J* 12.9 and 4.3 Hz, 5-H), 3.74 (1 H, d, *J* 8.6 Hz, 22-H), 4.00—4.26 (4 H, m, 2-H, 7-H_2, and 23-H), and 4.43 (1 H, m, $W_{\frac{1}{2}}$ 8.6 Hz, 3-H). Emitter CI-MS (isobutane), *m/z* 467 (M^+ + 1, base peak), 449, 431. EI-MS, *m/z* 433 (M^+ – 18 – 15), 430 (M^+ – 36), 415, 394, 380 (M^+ – 87, C_{22}–C_{23} fission, accompanied by H-transfer), 362 (base peak), 349, 344, 321 (C_{17}–C_{20} fission), 303, 285, 117, and 87 (Found: *m/z* 380.2564. Calc. for $C_{22}H_{26}O_5$: M^+ – 87, 380.2560).

(22R,23R,24S)-3α,22,23-*Trihydroxy*-5α-*ergostan*-6-*one* (*Typhasterol*) (2c).—The mesylate (15) (20 mg, 0.0375 mmol) was treated with lithium carbonate (5 mg, 0.0676 mmol) in dimethylformamide (5 ml) at 130 °C for 1 h. Work-up (ethyl acetate for extraction) gave a crude product (19 mg), which was applied to a column of silica gel (10 g). Elution with benzene provided the 2-ene (16) (9.2 mg, 52%), m.p. 235—237 °C (methanol), which was identical with the above prepared 2-ene (16) by treatment of (15) with lithium bromide, in terms of melting point, ^{1}H n.m.r. and mass spectra. Further elution with benzene–ethyl acetate (50 : 1) provided the 3α-formate (25) (5.0 mg, 26%), δ_H (100 MHz) 0.68 (3 H, s, 18-H$_3$), 1.35 (3 H, s, acetonide), 1.36 (3 H, s, acetonide), 3.70 (1 H, dd, *J* 8 and 4 Hz, 22-H or 23-H), 3.94 (1 H, d, *J* 8 Hz, 22-H or 23-H), 5.23 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 3β-H), and 8.01 (1 H, s, formyl). The 3α-formate (25) (5.0 mg, 0.00969 mmol) was refluxed with 70% aqueous acetic acid (5 ml) for 4 h. Removal of the solvent under reduced pressure gave the residue, which was then treated with 5% KOH–MeOH (3 ml) at room temperature for 1 h. Work-up (ethyl acetate for extraction) and chromatography on silica gel (5 g) with benzene–ethyl acetate (1 : 5) as eluant provided typhasterol (2c) (3.7 mg, 85%), m.p. 225—228.5 °C (aqueous acetonitrile), (lit.,[24] m.p. 227—230 °C), δ_H (360 MHz) 0.68 (3 H, s, 18-H$_3$), 0.73 (3 H, s, 19-H$_3$), 0.85 (3 H, d, *J* 6.7 Hz, 28-H$_3$), 0.91 (3 H, d, *J* 6.5 Hz, 21-H$_3$), 0.95 (3 H, d, *J* 6.7 Hz, 26-H$_3$), 0.98 (3 H, d, *J* 6.7 Hz, 27-H$_3$), 1.72 (1 H, dd, *J* 8.4 and 2.4 Hz), 2.31 (1 H, dd, *J* 12.7 and 4.8 Hz, 7β-H), 2.73 (1 H, seemingly t, *J* 7.7 Hz, 5α-H), 3.58 (1 H, d, *J* 8.4 Hz, 22-H), 3.72 (1 H, d, *J* 8.4 Hz, 23-H), and 4.17 (1 H, m, $W_{\frac{1}{2}}$ 6.7 Hz, 3β-H) (Found: *m/z* 448.3551. Calc. for $C_{28}H_{48}O_4$: M^+, 448.3554).

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