Synthesis and Activity of Plant Growth-promoting Steroids, (22*R*,23*R*,24*S*)-28-Homobrassinosteroids, with Modifications in Rings A and B

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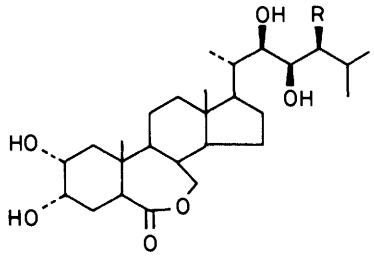
In order to investigate the structure-activity relationships of brassinosteroids, fourteen (22*R*,23*R*,24*S*)-28-homobrassinosteroids with modifications in rings A and B were synthesized from (22*E*,24*S*)-3β-hydroxy-5α-stigmast-22-en-6-one (3a). The (22*R*,23*R*)-vicinal diol was introduced by epoxidation of the *trans* C-22(23) double bond, followed by *trans* ring-opening of the resulting epoxides by HBr–AcOH, and then an inversion reaction at the carbon bearing bromine by acetoxy anion to give (22*R*,23*R*,24*S*)-3β,22,23-triacetoxy-5α-stigmastan-6-one (8a). Baeyer–Villiger oxidation of (8a) gave the two regioisomeric 6-oxa- and 7-oxa-lactones (15a) and (16a). Introduction of a 3α-hydroxy group and C-2(3) or C-3(4) double bond was achieved by treatment of the 3β-methanesulphonate (10b), (17b), or (25b) with lithium carbonate in refluxing dimethylformamide. Further elaboration of ring A gave 28-homobrassinosteroids, 2-deoxybrassinosteroids, 2-deoxy-4α-hydroxybrassinosteroids, and their regioisomeric 6-oxalactone compounds. Bioassay using the rice-lamina inclination test indicated that the 3α,4α-diol analogue (23) has almost the same activity as the 2α,3α-diol (1b), and the 2-deoxysteroids (13) and (21) were also highly active, showing one-tenth the activity of the 28-homobrassinolide (1b). The following structural features of brassinosteroids are important for the high activity: (i) the (22*R*,23*R*)-vicinal diol moiety; (ii) the (24*S*)-methyl or -ethyl group; (iii) the 7-oxalactone or 6-oxo functionality in the B-ring; (iv) a 3α-hydroxy group, 2α,3α-vicinal diol, or 3α,4α-vicinal diol; and (v) an A,B-*trans*-fused ring junction.

The discovery of the plant growth hormonal steroid brassinolide (1a), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one, from the pollen of rape (*Brassica napus* L.)[1] stimulated much effort on the part of plant physiologists and organic chemists to evaluate its activity and to achieve its synthesis and that of related compounds. When tested by the bean second-internode bioassay, brassinolide (1a) promoted both cell elongation and cell division at very low concentration;[1] it also showed a wide range of responses in a number of bioassay systems for auxin, gibberellin, and cytokinin.[2-6] We have already synthesized brassinolide (1a)[7,8] and many related compounds,[9-15] and have also clarified the structure-activity relationships of brassinosteroids, with particular emphasis on the stereochemical importance of the side chain.[13,15] Thompson *et al.* have also examined the structural requirements of brassinosteroids;[17,18] however, little is known about those of the A,B-ring functionalities. There is only one report examining this point,[19] in which several A-ring modified brassinosteroids with the unnatural (22*S*,23*S*)-vicinal diol were used for bioassay because of the unavailability of the natural (22*R*,23*R*)-isomers.

Brassinolide (1a) might be biosynthesized from 24-methylenecholesterol since this is the major sterol in the pollen.[1] From the biosynthetic point of view, conversion of the 3β-hydroxy group into the 2α,3α-vicinal diol is an interesting problem. Recently, (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (castasterone) (2a), a possible biosynthetic precursor of brassinolide (1a) was isolated and identified together with (1a) in some higher plants.[20,21] Therefore, it is possible that, as in the case of ecdysteroids,[22] A-ring modified biosynthetic precursors to brassinolide (1a) will be found which may have plant growth-promoting activity.[23]

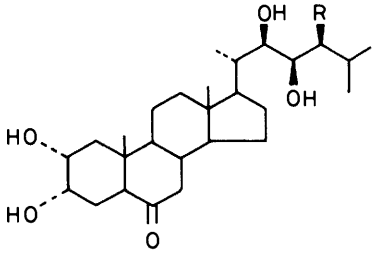
To gain further insight into the structural requirements for the A,B-ring part of brassinosteroids, we synthesized (22*R*,23*R*,24*S*)-28-homobrassinosteroids with modifications in rings A and B. We selected (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-stigmastan-6-one (28-homobrassinolide) (1b) as the standard compound instead of brassinolide (1a) itself for determination of the structure-activity relationships since this homologue is as highly active as brassinolide (1a) even at a concentration of 0.0001 p.p.m. in the rice-lamina inclination test,[13] is easily obtained from stigmasterol by our earlier reported synthesis,[10] and differs from brassinolide (1a) only in the 24-alkyl groups. In this paper we report the synthesis and plant growth-promoting



(1)

a: R = Me

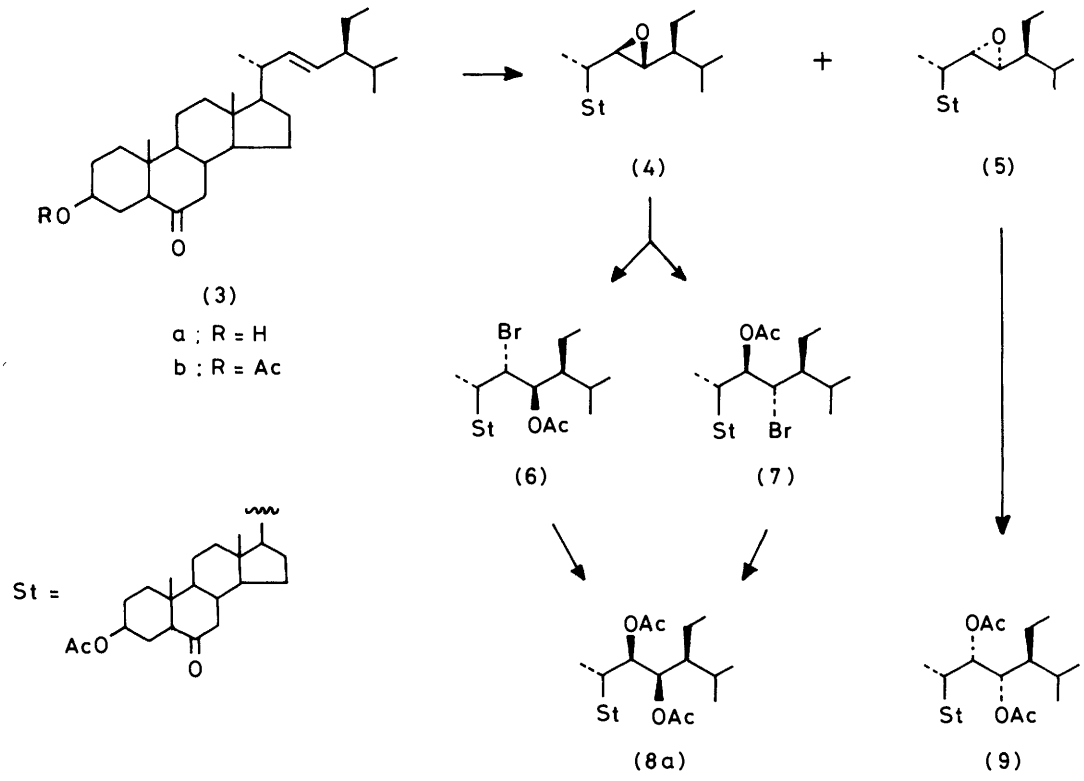
b: R = Et



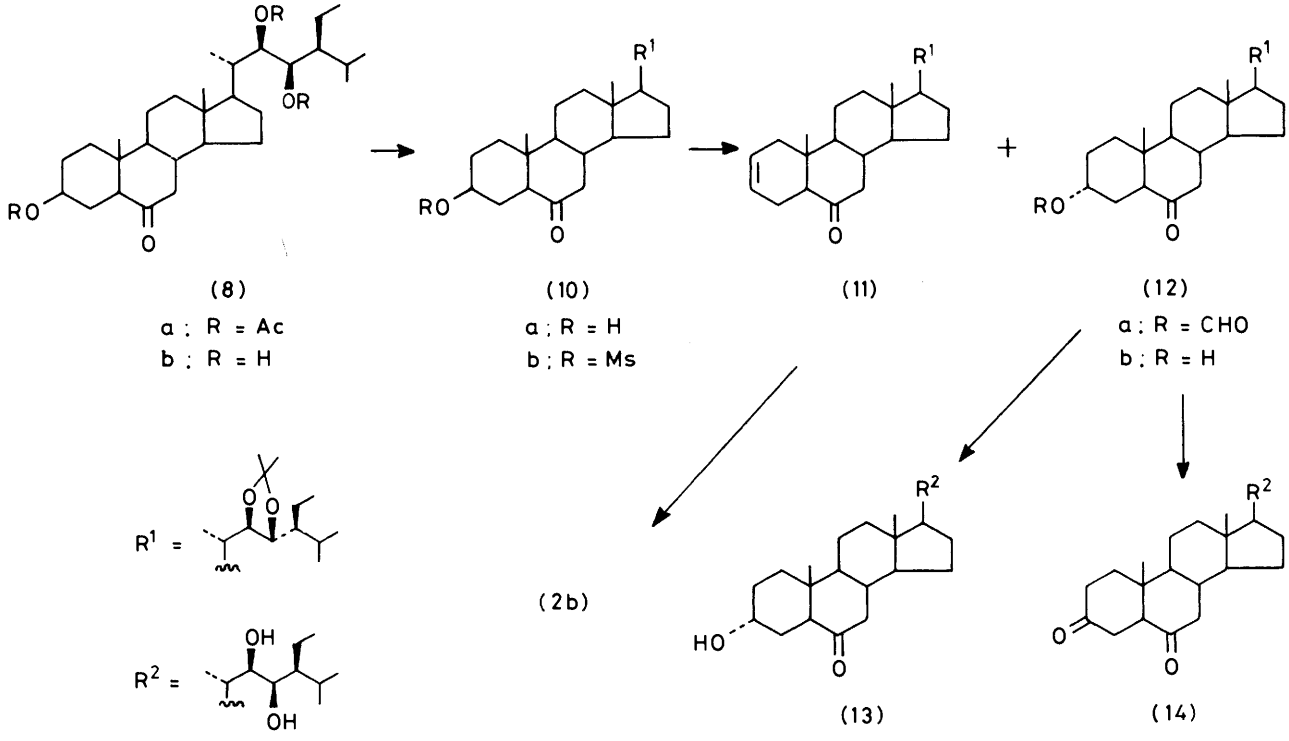
(2)

a: R = Me

b: R = Et



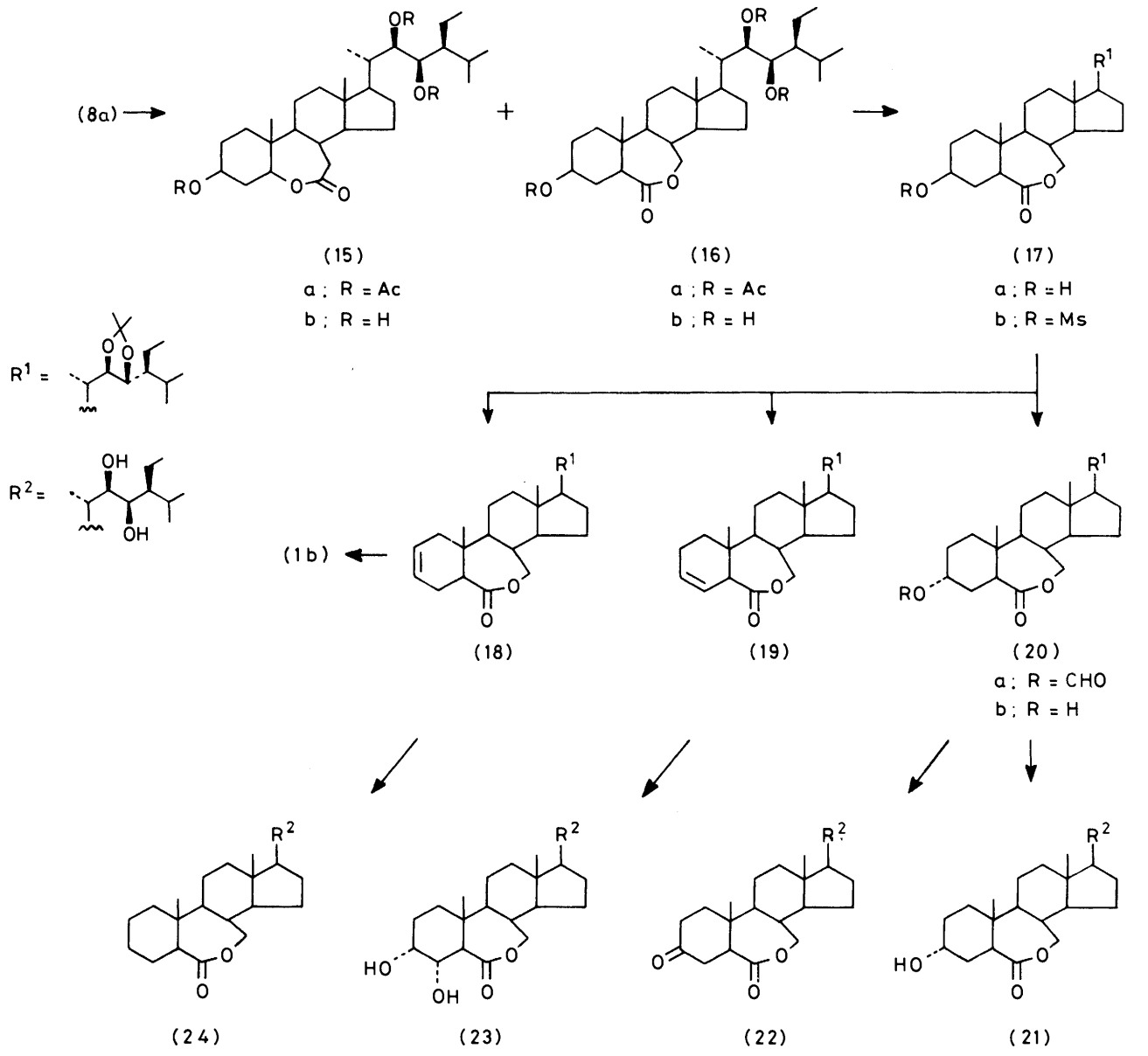
Scheme 1.



Scheme 2.

activity of the (22*R*,23*R*,24*S*)-28-homobrassinosteroids (1b), (2b), (8b), (13), (14), (15b), (16b), (21)—(24), and (29)—(31).

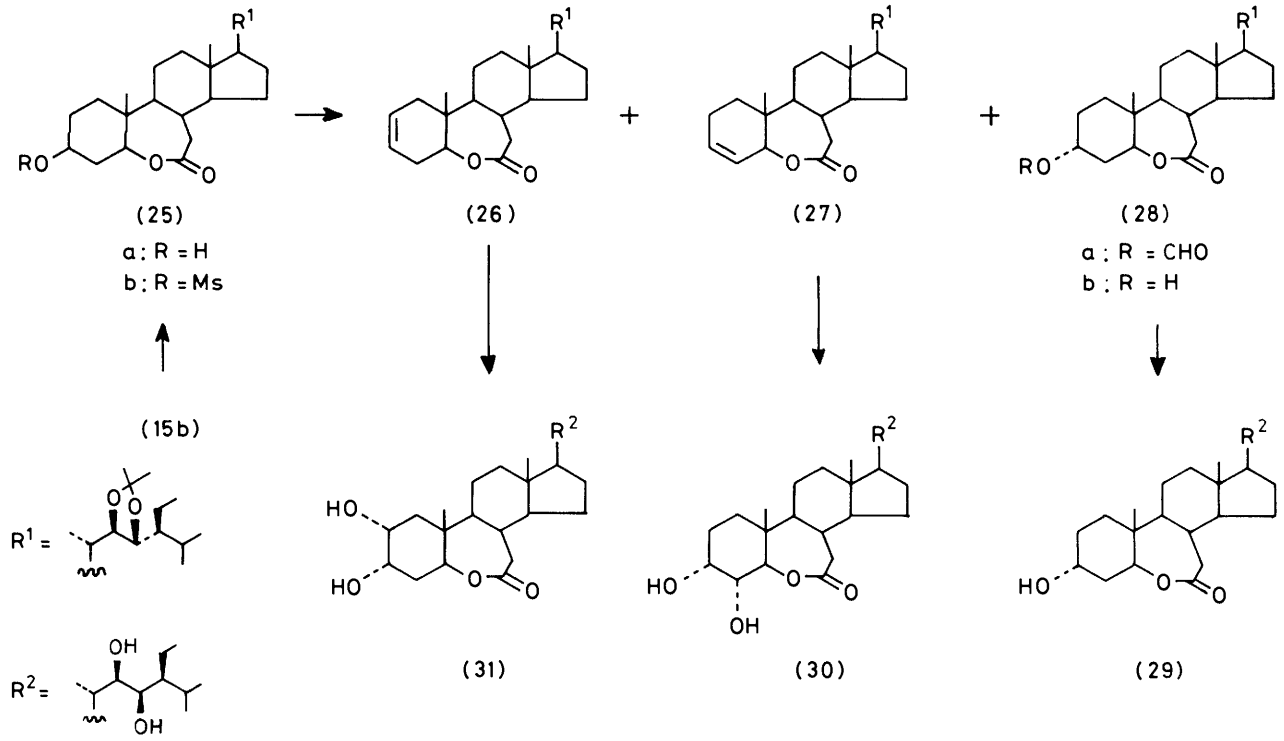
Our synthetic plan for the A,B-ring modified brassinosteroids is as follows; first, construction of the correct (22*R*,23*R*,24*S*)-configuration of the side chain, and then modification of rings A and B. Introduction of a (22*R*,23*R*)-vicinal diol function into the stigmasterol side chain was achieved by a slight modification of our previously reported method.[10]



Scheme 3.

The acetate (3b) of the previously described (22*E*,24*S*)-3β-hydroxy-5α-stigmastan-6-one (3a)[10] was treated with *m*-chloroperbenzoic acid to give a separable mixture of (22*R*,-23*R*)- and (22*S*,23*S*)-epoxides (Scheme 1). Chromatographic separation provided the less polar, major epoxide [59%, m.p. 139—140 °C, δ ($CDCl_3$) 2.70 (1 H, dd, *J* 8 and 2 Hz, 22- or 23-H)] and the more polar, minor epoxide [35%, m.p. 139—140 °C, δ ($CDCl_3$) 2.48 (2 H, m, 22- and 23-H)]. According to our previous examination of the epoxidation of stigmasterol derivatives,[24] the major (22*R*,23*R*)-epoxide showed a characteristic signal at δ 2.72 as a double doublet (*J* 8 and 2 Hz), while the minor (22*S*,23*S*)-isomer showed a signal at δ 2.46 as a multiplet. Therefore, the configuration of the less polar, major epoxide in the current study was assigned as (22*R*,23*R*) and that of the more polar, minor epoxide as (22*S*,23*S*). The major (22*R*,23*R*)-epoxide (4) was treated with 30% HBr–AcOH to give an inseparable mixture of the (22*S*,23*R*)- (6) and the (22*R*,23*S*)-bromoacetate (7). The mixture was treated with 80% aqueous acetic acid at 100 °C for 17 h and acetylated (at 60 °C for 15 h) to afford the (22*R*,23*R*,24*S*)-triacetoxy-6-oxosteroid (8a) in 35% yield; the stereochemistry at C-22 and -23 was confirmed by conversion of (8a) into the known (22*R*,23*R*,24*S*)-28-homobrassinolide (1b),[10] as described in the latter part of this paper. Therefore, the reaction was shown to proceed with inversion at the carbon bearing bromine. Since the (22*R*,23*R*,24*S*)-triacetate (8a) and the (22*S*,23*S*,24*S*)-triacetate (9) were found to differ greatly in their mobility on t.l.c. (see Experimental section) and these stereoisomers were easily separated by column chromatography, the mixture of the epoxides (4) and (5) was used directly in the above-described reactions. The (22*R*,23*R*,24*S*)-triacetate (8a) (34%) and the (22*S*,23*S*,24*S*)-isomer (9) (22%) were obtained without separation of any intermediates.

Modification of rings A and B of the (22*R*,23*R*,24*S*)-6-oxosteroid were then carried out (Scheme 2). Saponification of (8a) provided (22*R*,23*R*,24*S*)-3β,22,23-trihydroxy-5α-stigmastan-6-one (8b), m.p. 206—209 °C. This was converted into the isopropylidene derivative (10a), which was treated with



Scheme 4.

methanesulphonyl chloride–pyridine to provide the 3β-methanesulphonate (10b). Treatment of (10b) in dimethylformamide with lithium carbonate under reflux provided the 2-ene (11) [35% from (8a), m.p. 238—241 °C], the 3α-formate (12a) [22% from (8a), m.p. 188—190 °C, δ ($CDCl_3$) 5.23 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H), 8.01 (1 H, s, formyl)], and the mixture of the 3α-ol (12b) and the 3β-ol (10a) [20% from (8a)]. Removal of the protecting groups of (12a) with 80% aqueous acetic acid and 5% KOH–MeOH afforded (22*R*,23*R*,24*S*)-3α,22,23-trihydroxy-5α-stigmastan-6-one (13), m.p. 239—240 °C, in 98% yield. Oxidation of the mixture of (12b) and (10a) with pyridinium chlorochromate in dichloromethane and removal of the protecting group gave (22*R*,23*R*,24*S*)-22,23-dihydroxy-5α-stigmastane-3,6-dione (14), m.p. 196—198 °C, in 90% yield. Stereospecific α-face hydroxylation of the 2-ene (11) with a catalytic amount of osmium tetraoxide and *N*-methylmorpholine *N*-oxide in Bu^{t}OH–tetrahydrofuran (THF)–H_2O (10 : 3 : 1) and deprotection afforded the known (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-stigmastan-6-one (2b), m.p. 253—256 °C (lit.,[10] m.p. 253—255 °C), in 93% yield.

The (22*R*,23*R*,24*S*)-triacetoxy-6-oxosteroid (8a) was subjected to Baeyer–Villiger oxidation (Scheme 3). Treatment of (8a) with trifluoroperacetic acid in dichloromethane in the presence of disodium hydrogen phosphate at 0 °C for 2 h provided the less polar 7-oxalactone (16a) (56%) [δ ($CDCl_3$) 2.87 (1 H, dd, *J* 9 and 8 Hz, 5α-H), 4.01 (2 H, m, 7-H_2)] and the more polar 6-oxolactone (15a) (36%) [δ ($CDCl_3$) 4.27 (1 H, dd, *J* 12 and 6 Hz, 5α-H), 2.42 (2 H, m, 7-H_2)], after chromatographic purification.[25] The acetate (16a) was saponified and subsequently treated with acid to give (22*R*,23*R*,24*S*)-3β,22,23-trihydroxy-B-homo-7-oxa-5α-stigmastan-6-one (16b), m.p. 186—189 °C. This was converted into the isopropylidene derivative, and then methanesulphonated to give the methanesulphonate (17b). Treatment of (17b) with lithium carbonate in refluxing dimethylformamide provided the 2-ene (18) [24% from (16a), m.p. 227—229 °C, δ ($CDCl_3$) 2.90 (1 H, m, 5α-H), 5.57 (2 H, m, 2- and 3-H)], the 3-ene (19) [18% from (16a), m.p. 233—235 °C, δ ($CDCl_3$) 3.50 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 5a-H), 5.40—6.13 (2 H, m, 3- and 4-H)], and the 3α-formate (20a) [28% from (16a), m.p. 220—223 °C, δ ($CDCl_3$) 5.22 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 3β-H), 8.02 (1 H, s, formyl)], accompanied by a separable mixture of the 3α-ol (20b) [17% from (16a), m.p. 232—232.5 °C] and the 3β-ol (17a) [5% from (16a)]. The formation of the 3-ene (19) in the case of the 7-oxalactone (17b) is noteworthy; such a formation was not observed in the case of the 6-oxosteroids (10b). Removal of the protecting groups of (20a) afforded (22*R*,23*R*,24*S*)-3α,22,23-trihydroxy-B-homo-7-oxa-5α-stigmastan-6-one (21), m.p. 216—217 °C, in 93% yield. Oxidation of the 2-ene (18) with osmium tetraoxide and removal of the protecting group provided the known (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-stigmastan-6-one (1b) [95%, m.p. 268—272 °C, lit.,[10] m.p. 268—271 °C]. The i.r. and mass spectra and the mobility on t.l.c. of the synthetic (22*R*,23*R*,24*S*)-28-homobrassinolide (1b) were identical with those of the authentic sample.[10] Similarly, the 3-ene (19) was converted into (22*R*,23*R*,24*S*)-3α,4α,22,23-tetrahydroxy-B-homo-7-oxa-5α-stigmastan-6-one (23), m.p. 268—269 °C, in 90% yield. In its ^1H n.m.r. spectrum, (23) showed characteristic signals at δ 3.00 (1 H, d, *J* 10 Hz, 5α-H) and 4.42 (1 H, dd, *J* 10 and 3 Hz, 4β-H), which strongly support the assigned structure. Hydrogenation of the 2-ene (18) and deprotection gave (22*R*,23*R*,24*S*)-22,23-dihydroxy-B-homo-7-oxa-5α-stigmastan-6-one (24), as an amorphous solid, in 96% yield. The mixture of the 3α-ol (20b) and the 3β-ol (17a) was oxidized and deprotected, as described for (14), to afford (22*R*,23*R*,24*S*)-22,23-dihydroxy-B-homo-7-oxa-5α-stigmastane-3,6-dione (22), m.p. 270—272 °C.

The regioisomeric 6-oxalactone analogues (15b) and (29)—(31) were synthesized from the triacetoxy-6-oxalactone (15a) as follows (Scheme 4). The acetate (15a) was saponified and acidified to provide (22*R*,23*R*,24*S*)-3β,22,23-trihydroxy-B-homo-6-oxa-5α-stigmastan-7-one (15b), m.p. 255—256 °C, which was converted into the methanesulphonate (25b). Treatment of (25b) with lithium carbonate in refluxing dimethylformamide also gave the 3-ene (27) [14% from (15a),

m.p. 228—229 °C, δ ($CDCl_3$) 4.75 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 5α-H), 5.30—6.00 (2 H, m, 3- and 4-H)], as in the case of the regio-isomeric 7-oxalactone (17b), together with the 2-ene (26) [26% from (15a), m.p. 195—197 °C, δ ($CDCl_3$) 4.42 (1 H, dd, *J* 9 and 8 Hz, 5α-H), 5.50 (2 H, m, 2- and 3-H)], the 3α-formate (28a) [13% from (15a), an oil, δ ($CDCl_3$) 4.48 (1 H, dd, *J* 12 and 6 Hz, 5α-H), 5.27 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H), 8.01 (1 H, s, formyl)], and the 3α-ol (28b) [11% from (15a), an oil, δ ($CDCl_3$) 4.18 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 3-H), 4.60 (1 H, dd, *J* 12 and 6 Hz, 5α-H)]. The 3α-formate (28a) and the 3α-ol (28b) were deprotected to give (22*R*,23*R*,24*S*)-3α,22,23-trihydroxy-B-homo-6-oxa-5α-stigmastan-7-one (29), m.p. 239—240 °C, in 92% yield. The 2-ene (26) and the 3-ene (27) were converted, as described for (1b) and (23), into (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-B-homo-6-oxa-5α-stigmastan-7-one (31), m.p. 277—279 °C, and (22*R*,23*R*,24*S*)-3α,4α,22,23-tetrahydroxy-B-homo-6-oxa-5α-stigmastan-7-one (30), m.p. 254—255 °C, respectively.

The plant growth-promoting activities of our synthetic brassinosteroids, (1b), (2b), (8b), (13), (14), (15b), (16b), (21)—(24), (29)—(31), were examined using the rice-lamina inclination test, which has recently been found to be a useful bioassay for evaluation of the activity of brassinosteroids.[6,13,19] As expected, 28-homobrassinolide (1b) was found to be the most active of the analogues tested, while its isomeric 6-oxalactone (31) was *ca.* one-hundredth as active, indicating that the 7-oxalactone functionality of (1a) cannot be replaced by the isomeric 6-oxalactone functionality. (2b) and (23) showed *ca.* one-half the activity of (1b). It is of interest that the 2-deoxysteroids (13) and (21) were also highly active and showed *ca.* one-tenth the activity of (1b). These results indicate for the first time that the 2α,3α-vicinal diol is not essential for the activity, although it is decreased when the group is not present. The other analogues showed *ca.* 1—10% of the activity of (1b) in this bioassay.

The present investigation of the structure-activity relationship of brassinosteroids has shown for the first time that rings A and B can be modified without changing the activity if the correct natural (22*R*,23*R*,24*S*)-configuration in the side chain is retained. We have already noted [13,15] the stereochemical importance of the (22*R*,23*R*)-vicinal diol and the (24*S*)-methyl or -ethyl group. Therefore, we conclude that the following structural features of brassinosteroids are important for high activity: (i) a (22*R*,23*R*)-vicinal diol moiety; (ii) a (24*S*)-methyl or -ethyl group; (iii) a 7-oxalactone or 6-oxo functionality in the B-ring; (iv) a 3α-hydroxy group, a 2α,3α-vicinal diol, or a 3α,4α-vicinal diol; and (v) an A,B-*trans*-fused ring junction.

Experimental

M.p.s were determined by a hot-stage microscope apparatus and were uncorrected. ^1H N.m.r. spectra were recorded with a Hitachi R-24A (60 MHz) or JEOL PS 100 (100 MHz) spectrometer in $CDCl_3$ solution with tetramethylsilane as an internal standard unless otherwise stated. I.r. spectra were recorded with a Hitachi Model 260-10 spectrometer. Mass spectra were taken with a Hitachi M-80 mass spectrometer at 70 eV. Kiesel gel 60 F_{254} (Merck) was used for analytical and preparative t.l.c. Column chromatography was effected with Kiesel gel 60 (70—230 mesh, Merck). The usual work-up refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying ($MgSO_4$), and removal of the solvent under reduced pressure. Ether refers to diethyl ether. The following abbreviations are used in the ^1H n.m.r. and i.r. spectral assignments: s, singlet; d, doublet; dd, double doublet; bs, broad singlet; m, multiplet; and s, strong; m, medium; w, weak; sh, shoulder, respectively.

(22E,24S)-3β-*Acetoxy*-5α-*stigmast*-22-*en*-6-*one* (3b).—The known (22*E*,24*S*)-3β-hydroxy-5α-stigmast-22-en-6-one (3a) [10] (10 g, 23.36 mmol) was treated with acetic anhydride (25 ml) and pyridine (50 ml) at room temperature for 18 h. The usual work-up (ethyl acetate for extraction) provided the 3β-*acetate* (3b) (10.9 g, 99%), m.p. 146—147.5 °C (from methanol), δ ($CDCl_3$) 0.67 (3 H, s, 18-H_3), 0.77 (3 H, s, 19-H_3), 2.01 (3 H, s, acetyl), 4.64 (1 H, m, 3α-H), and 5.05 (2 H, m, 22- and 23-H) (Found: C, 79.0; H, 10.8. $C_{31}H_{50}O_3$ requires C, 79.10; H, 10.71%).

(22R,23R,24S)-3β-*Acetoxy*-22,23-*epoxy*-5α-*stigmastan*-6-*one* (4) *and* (22S,23S,24S)-3β-*Acetoxy*-22,23-*epoxy*-5α-*stigmastan*-6-*one* (5).—The 22*E*-olefin (3b) (6.2 g, 13.19 mmol) in dichloromethane (50 ml) was treated with *m*-chloroperbenzoic acid (2.5 g, 14.45 mmol) at room temperature for 15 h. To this reaction mixture, calcium hydroxide (4 g) was added. This mixture was stirred at room temperature for 1 h. Filtration and removal of the solvent gave a separable mixture of two products (6.1 g), which was applied to a column of silica gel (100 g). Elution with benzene–ethyl acetate (50 : 1) provided the less polar, major (22R,23R)-*epoxide* (4) (3.8 g, 59%), m.p. 139—140 °C (from methanol), R_F 0.44 (benzene–ethyl acetate, 10 : 1, developed once), δ ($CDCl_3$) 0.67 (3 H, s, 18-H_3), 0.77 (3 H s, 19-H_3), 2.02 (3 H, s, acetyl), 2.70 (1 H, dd, *J* 8 and 2 Hz, 22- or 23-H), and 4.65 (1 H, m, 3α-H) (Found: C, 76.4; H, 10.4. $C_{31}H_{50}O_4$ requires C, 76.50; H, 10.36%).

Further elution with the same solvent provided the more polar, minor (22S,23S)-*epoxide* (5) (2.3 g, 35%), m.p. 139—140 °C (from methanol), R_F 0.37 (benzene–ethyl acetate, 10 : 1, developed once), δ ($CDCl_3$) 0.66 (3 H, s, 18-H_3), 0.76 (3 H, s, 19-H_3), 2.01 (3 H, s, acetyl), 2.48 (2 H, m, 22- and 23-H), and 4.65 (1 H, m, 3α-H) (Found: C, 76.45; H, 10.5. $C_{31}H_{50}O_4$ requires C, 76.50; H, 10.36%).

(22R,23R,24S)-3β,22,23-*Triacetoxy*-5α-*stigmastan*-6-*one* (8a).—The (22*R*,23*R*)-epoxide (4) (1.7 g, 3.50 mmol) in acetic acid (10 ml) was treated with 30% HBr–AcOH (8 ml) at room temperature for 6 h. The usual work-up (ethyl acetate for extraction) provided a 1 : 2 mixture of the bromoacetates (6) and (7) (2.1 g); δ ($CDCl_3$) 0.64 (2 H, s, 18-H_3), 0.70 (1 H, s, 18-H_3), 0.77 (3 H, s, 19-H_3), 2.02 (3 H, s, acetyl), 2.04 (2 H, s, acetyl), 2.07 (1 H, s, acetyl), 4.20 ($\frac{1}{3}$H, d, *J* 10 Hz), 4.38 ($\frac{2}{3}$H, d, *J* 10 Hz), 4.65 (1 H, m, 3α-H), and 5.38 (1 H, d, *J* 10 Hz). This mixture of bromoacetates was treated with acetic acid (50 ml) and water (10 ml) at 100 °C for 19 h. Removal of the solvent under reduced pressure gave the residue, which was acetylated with acetic anhydride (10 ml) and pyridine (10 ml) at 60 °C for 15 h. The usual work-up (ethyl acetate for extraction) provided the crude product (1.9 g), which was applied to a column of silica gel (50 g). Elution with benzene–ethyl acetate (25 : 2) provided the *triacetoxy*-6-*oxosteroid* (8a) (720 mg, 35%), m.p. 204—205 °C (from methanol); δ ($CDCl_3$) 0.66 (3 H, s, 18-H_3), 0.75 (3 H, s, 19-H_3), 1.95 (3 H, s, acetyl), 2.00 (6 H, s, 2 acetyls), 4.65 (1 H, m, 3α-H), 5.09 (1 H, d, *J* 9.5 Hz, 22- or 23-H), and 5.29 (1 H, d, *J* 9.5 Hz, 22- or 23-H) (Found: C, 71.3; H, 9.6. $C_{35}H_{56}O_7$ requires C, 71.39; H, 9.59%).

(22R,23R,24S)-3β,22,23-*Triacetoxy*-5α-*stigmastan*-6-*one* (8a) *and* (22S,23S,24S)-3β,22,23-*Triacetoxy*-5α-*stigmastan*-6-*one* (9).—A mixture of the (22*R*,23*R*)- and (22*S*,23*S*)-epoxides (4) and (5) (1.25 g, 2.57 mmol), which was obtained by epoxidation of (3a) as described for (4), was treated as described for (8a) to give a mixture of the (22*S*,23*S*)-triacetate (9) (R_F 0.47, benzene–ethyl acetate, 10 : 1, developed once) and the (22*R*,23*R*)-triacetate (8a) (R_F 0.28, the same solvent system). This mixture (1.26 g) was applied to a column of

silica gel (50 g). Elution with benzene–ethyl acetate (25 : 2) provided the less polar, minor (22S,23S)-*triacetate* (9) (340 mg, 22%), m.p. 170—172 °C (from methanol), δ ($CDCl_3$) 0.65 (3 H, s, 18-H_3), 0.75 (3 H, s, 19-H_3), 2.01 (3 H, s, acetyl), 2.03 (3 H, s, acetyl), 2.07 (3 H, s, acetyl), 4.65 (1 H, m, 3α-H), 5.00 (1 H, m, 22- or 23-H), and 5.20 (1 H, m, 22- or 23-H) (Found: C, 71.3; H, 9.7. $C_{35}H_{56}O_7$ requires C, 71.39; H, 9.59%). Further elution with the same solvent provided the more polar, major (22*R*,23*R*)-triacetate (8a) (520 mg, 34%), which was identical (m.p., ^1H n.m.r., t.l.c.) with the sample obtained above.

(22R,23R,24S)-3β,22,23-*Trihydroxy*-5α-*stigmastan*-6-*one* (8b).—The triacetate (8a) (520 mg, 0.861 mmol) was saponified with 5% KOH–MeOH (10 ml) under reflux for 1 h. The usual work-up (ethyl acetate for extraction) provided the *trihydroxy*-6-*oxosteroid* (8b) (390 mg, 98%), m.p. 206—209 °C (from ethyl acetate–hexane); δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 0.75 (3 H, s, 19-H_3), 3.52 (1 H, m, 3α-H), 3.58 (1 H, d, *J* 9 Hz, 22-H), and 3.72 (1 H, d, *J* 9 Hz, 23-H) (Found: C, 75.05; H, 10.7. $C_{29}H_{50}O_4$ requires C, 75.28; H, 10.89%).

(22R,23R,24S)-3β-*Hydroxy*-22,23-*isopropylidenedioxy*-5α-*stigmastan*-6-*one* (10a).—The triol (8b) (450 mg, 0.974 mmol) in acetone (30 ml) was treated with toluene-*p*-sulphonic acid (10 mg) at room temperature for 2 h. The usual work-up (ether extraction) provided the acetonide (10a) (489 mg); δ ($CDCl_3$) 0.64 (3 H, s, 18-H_3), 0.74 (3 H, s, 19-H_3), 1.35 (6 H, s, acetonide), 3.52 (1 H, m, 3α-H), and 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-3β-*methylsulphonyloxy*-5α-*stigmastan*-6-*one* (10b).—The 3β-ol (10a) (489 mg) was treated with methanesulphonyl chloride (0.5 ml) and pyridine (3 ml) at room temperature for 1 h. To this reaction mixture, ice was added. The usual work-up (ethyl acetate extraction) provided the *methanesulphonate* (10b) (513 mg); δ ($CDCl_3$) 0.64 (3 H, s, 18-H_3), 0.73 (3 H, s, 19-H_3), 1.34 (6 H, s, acetonide), 3.00 (3 H, s, mesyl), 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), and 4.53 (1 H, m, 3α-H).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-5α-*stigmast*-2-*en*-6-*one* (11) *and* (22R,23R,24S)-3α-*Formyloxy*-22,23-*isopropylidenedioxy*-5α-*stigmastan*-6-*one* (12a).—The mesylate (10b) (513 mg) in dimethylformamide (10 ml) was treated with lithium carbonate (350 mg, 4.73 mmol) under reflux for 1 h. The usual work-up (ethyl acetate extraction) gave a crude product (423 mg), which was applied to a column of silica gel (20 g). Elution with benzene–ethyl acetate (100 : 1) provided the 2-*ene* (11) [105 mg, 25% from (8a)], m.p. 238—241 °C (from methanol), δ ($CDCl_3$) 0.66 (3 H, s, 18-H_3), 0.69 (3 H, s, 19-H_3), 1.33 (6 H, s, acetonide), 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), and 5.58 (2 H, m, 2- and 3-H) (Found: C, 79.2; H, 10.9. $C_{32}H_{52}O_3$ requires C, 79.28; H, 10.81%). Further elution with benzene–ethyl acetate (50 : 1) gave the 3α-*formate* (12a) [99 mg, 22% from (8a)], m.p. 188—190 °C (from methanol); δ ($CDCl_3$) 0.66 (3 H, s, 18-H_3), 0.75 (3 H, s, 19-H_3), 1.35 (6 H, s, acetonide), 3.76 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 5.23 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H), and 8.01 (1 H, s, formyl) (Found: C, 74.6; H, 10.3. $C_{33}H_{54}O_5$ requires C, 74.67; H, 10.26%). Further elution with ethyl acetate provided a mixture of the 3α-ol (12b) and the 3β-ol (10a) [85 mg, 20% from (8a)].

(22R,23R,24S)-3α,22,23-*Trihydroxy*-5α-*stigmastan*-6-*one* (13).—Compound (12a) (90 mg, 0.170 mmol) was treated with acetic acid (9 ml) and water (1 ml) under reflux for 5 h. Removal of the solvent under reduced pressure gave the residue (86 mg), which was saponified with 5% KOH–MeOH (5 ml) under reflux for 15 min. The usual work-up (ethyl acetate for extraction) gave the *trihydroxy*-6-*oxosteroid* (13) (77 mg, 98%), m.p. 239—240 °C (from ethyl acetate), δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 0.76 (3 H, s, 19-H_3), 2.76 (1 H, dd, *J* 13 and 5 Hz, 5α-H), 3.58 (1 H, d, *J* 9 Hz, 22-H), 3.72 (1 H, d, *J* 9 Hz, 23-H), and 4.17 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H) (Found: C, 75.1; H, 10.9. $C_{29}H_{50}O_4$ requires C, 75.28; H, 10.89%).

(22R,23R,24S)-22,23-*Dihydroxy*-5α-*stigmastane*-3,6-*dione* (14).—The mixture of the 3α-ol (12b) and the 3β-ol (10a) (40 mg, 0.0797 mmol) in dichloromethane (3 ml) was treated with pyridinium chlorochromate (20 mg, 0.093 mmol) at room temperature for 2 h. To this reaction mixture, ether (20 ml) was added. Filtration through a Florisil column and removal of the solvent provided (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy-5α-stigmastane-3,6-dione (38 mg), m.p. 217—220 °C (from methanol); δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 3.76 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H); high resolution mass spectrum *m/z* 500.3867 (M^+, calc. for $C_{32}H_{52}O_4$: *m/z* 500.3868). This acetonide (38 mg) was treated with 70% aqueous acetic acid (6 ml) under reflux for 6 h. Removal of the solvent under reduced pressure and preparative t.l.c. (benzene–ethyl acetate, 1 : 1, developed once) provided the 3,6-*diketone* (14) (25 mg, 68%), m.p. 196—198 °C (from ethyl acetate–hexane); δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 3.58 (1 H, d, *J* 9 Hz, 22-H), and 3.72 (1 H, d, *J* 9 Hz, 23-H) (Found: C, 75.4; H, 10.7. $C_{29}H_{48}O_4$ requires C, 75.60; H, 10.50%).

(22R,23R,24S)-2α,3α,22,23-*Tetrahydroxy*-5α-*stigmastan*-6-*one* (2b).—The 2-ene (11) (25 mg, 0.0517 mmol) in t-butyl alcohol–THF–H_2O (10 : 3 : 1; 5 ml) was treated with osmium tetraoxide (2 mg) and *N*-methylmorpholine *N*-oxide (50 mg) at room temperature for 16 h. To this reaction mixture, satd. $NaHSO_3$ solution (10 ml) was added. The mixture was stirred at room temperature for 2 h. The usual work-up (dichloromethane extraction) gave a crude product (26 mg), which was treated with acetic acid (5 ml) and water (1 ml) under reflux for 5 h. Removal of the solvent under reduced pressure gave the crude product (25.5 mg). This was applied to a column of silica gel (5 g). Elution with ethyl acetate provided the tetraol (2b) (23.5 mg, 95%), m.p. 253—256 °C (lit.,[10] m.p. 253—255 °C) (from ethyl acetate), $\nu_{max.}$ (KBr) 3 440 (s), 2 948 (s), 2 910 (sh), 2 875 (s), 1 710 (s), 1 690 (sh), 1 642 (w), 1 467 (m), 1 390 (m), 1 370 (sh), 1 340 (w), 1 330 (w), 1 312 (w), 1 280 (m), 1 262 (w), 1 237 (w), 1 130 (w), 1 010 (w), 1 085 (m), 1 060 (sh), 1 045 (m), 1 019 (m), and 993 cm^{-1} (w); field desorption mass spectrum *m/z* 479 ($M^+ + 1$), 451 ($M^+ + 1 - 18$), 393 [$M^+ -$ 85, C(23)–C(24) cleavage], 363 [$M^+ - 115$, C(22)–C(24) cleavage], 333[$M^+ - 145$, C(20)–C(22) cleavage], 145, 115, and 85 (Found: C, 72.7; H, 10.6. Calc. for $C_{29}H_{50}O_5$: C, 72.76; H, 10.53%).

(22R,23R,24S)-3β,22,23-*Triacetoxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (16a) *and* (22R,23R,24S)-3β,22,23-*Triacetoxy* B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (15a).—The triacetoxy-6-oxosteroid (8a) (650 mg, 1.11 mmol) in dichloromethane (10 ml) was treated with trifluoroperacetic acid (7 equiv.) in the presence of disodium hydrogen phosphate (2 g) at 0 °C for 3 h. The usual work-up (ethyl acetate extraction) gave a crude product (655 mg), which was applied to a column of silica gel (50 g). Elution with hexane–ethyl acetate (2 : 1) provided the less polar, major 7-oxalactone (16a) (362 mg, 56%), as an oil, R_F 0.49 (benzene–ethyl acetate, 3 : 1, developed once), δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 0.89 (3 H, s, 19-H_3), 1.95 (3 H, s, acetyl), 2.00 (6 H, s, 2 acetyls), 2.87 (1 H, dd, *J* 9 and 8 Hz, 5α-H), 4.01 (2 H, m, 7-H_2), 4.60 (1 H, m, 3α-H), 5.09 (1 H, d, *J* 9.5 Hz, 22- or 23-H), and 5.29 (1 H, d, *J* 9.5 Hz,

22- or 23-H). Further elution with the same solvent gave the more polar, minor 6-oxalactone (15a) (234 mg, 36%), as an oil, R_F 0.42 (benzene–ethyl acetate, 3 : 1, developed once), δ ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 0.92 (3 H, s, 19-H_3), 1.95 (3 H, s, acetyl), 2.00 (3 H, s, acetyl), 2.02 (3 H, s, acetyl), 2.42 (2 H, m, 7-H_2), 4.27 (1 H, dd, *J* 12 and 6 Hz, 5α-H), 4.70 (1 H, m, 3β-H), 5.12 (1 H, d, *J* 9.5 Hz, 22- or 23-H), 5.31 (1 H, d, *J* 9.5 Hz, 22- or 23-H).

(22R,23R,24S)-3β,22,23-*Trihydroxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (16b).—The triacetate (16a) (362 mg, 0.599 mmol) was treated with 5% KOH–MeOH (15 ml) under reflux for 1 h. After being cooled to room temperature, 6M-HCl (15 ml) was added to the reaction mixture and it was stirred at room temperature for 1 h. The usual work-up (ethyl acetate extraction) provided the *trihydroxy*-7-*oxalactone* (16b) (273 mg, 95%), m.p. 186—189 °C (from ethyl acetate), δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 2.84 (1 H, dd, *J* 8.5 and 8 Hz, 5α-H), 3.48 (1 H, m, 3α-H), 3.58 (1 H, d, *J* 9 Hz, 22-H), 3.72 (1 H, d, *J* 9 Hz, 23-H), and 4.10 (2 H, m, 7-H_2) (Found: C, 72.7; H, 10.6. $C_{29}H_{50}O_5$ requires C, 72.76; H, 10.53%).

(22R,23R,24S)-3β-*Hydroxy*-22,23-*isopropylidenedioxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (17a).—The triol (16b) (216 mg, 0.452 mmol) in acetone (20 ml) was treated with toluene-*p*-sulphonic acid (20 mg) at room temperature for 18 h. The usual work-up (ether extraction) gave the product (17a) (234 mg), δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 1.34 (6 H, s, acetonide), 2.84 (1 H, dd, *J* 8.5 and 8 Hz, 5α-H), 3.48 (1 H, m, 3α-H), 3.76 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), and 4.02 (2 H, m, 7-H_2).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-3β-*methylsulphonyloxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (17b).—The 3β-ol (17a) (234 mg) in pyridine (3 ml) was treated with methanesulphonyl chloride (0.5 ml) at room temperature for 4 h. To this reaction mixture ice was added. The usual work-up (ethyl acetate extraction) provided the *methanesulphonate* (17b) (266 mg), δ ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 2.89 (1 H, dd, *J* 9 and 8 Hz, 5α-H), 3.01 (3 H, s, mesyl), 3.77 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 4.02 (2 H, m, 7-H_2), and 4.54 (1 H, m, 3α-H).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-B-*homo*-7-*oxa*-5α-*stigmast*-2-*en*-6-*one* (18).—The methanesulphonate (17b) (266 mg) was treated with lithium carbonate (150 mg, 1.62 mmol) as described for (10b). The usual work-up and chromatography on silica gel (30 g) eluting with benzene–ethyl acetate (50 : 1) provided the 2-*ene* (18) [54 mg, 24% from (16a)], m.p. 227—229 °C (from methanol), R_F 0.63 (benzene–ethyl acetate, 10 : 1, developed once), δ ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 1.34 (6 H, s, acetonide), 2.90 (1 H, m, 5α-H), 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 4.05 (2 H, m, 7-H_2), and 5.57 (2 H, m, 2- and 3-H) (Found: C, 76.6; H, 10.5. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.47%).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-B-*homo*-7-*oxa*-5α-*stigmast*-3-*en*-6-*one* (19).—Further elution of the above mixture with the same solvent provided the 3-*ene* (19) [40 mg, 18% from (16a)], m.p. 233—235 °C (from methanol), R_F 0.57 (benzene–ethyl acetate, 10 : 1, developed once), δ ($CDCl_3$) 0.71 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 3.50 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 5α-H), 3.76 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 4.10 (2 H, m, 7-H_2), and 5.40—6.13 (2 H, m, 3- and 4-H) (Found: C, 76.6; H, 10.5. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.47%).

(22R,23R,24S)-3α-*Formyloxy*-22,23-*isopropylidenedioxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (20a).—Further elution of the above mixture with benzene–ethyl acetate (20 : 1) provided the 3α-*formate* (20a) [70 mg, 28% from (16a)], m.p. 220—223 °C (from methanol), R_F 0.38 (benzene–ethyl acetate, 10 : 1, developed once), δ ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 3.05 (1 H, dd, *J* 13 and 6 Hz, 5α-H), 3.77 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 4.08 (2 H, m, 7-H_2), 5.22 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 3β-H), and 8.02 (1 H, s, formyl) (Found: C, 72.45; H, 10.0. $C_{33}H_{54}O_6$ requires C, 72.49; H, 9.96%).

(22R,23R,24S)-3α-*Hydroxy*-22,23-*isopropylidenedioxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (20b).—Further elution with ethyl acetate gave a mixture of the 3α-ol (20b) and the 3β-ol (17a) (60 mg). Preparative t.l.c. (chloroform–methanol, 20 : 1, developed twice) provided the less polar 3α-*ol* (20b) [42 mg, 17% from (16a)], m.p. 232—232.5 °C (from methanol), R_F 0.59, δ ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 3.18 (1 H, dd, *J* 12 and 6 Hz, 5α-H), 3.77 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), and 3.93—4.30 (3 H, m, 3β-H and 7-H_3); high resolution mass spectrum, *m/z* 518.3977 (M^+, $C_{32}H_{54}O_5$ requires *m/z* 518.3973). The acetate of (20b) had δ ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 2.05 (3 H, s, acetyl), 3.00 (1 H, dd, *J* 11 and 5 Hz, 5α-H), 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 4.05 (2 H, m, 7-H_2), and 5.06 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H); and the 3β-ol (17a) [12 mg, 5% from (16a)], identical (^{1}H n.m.r., t.l.c.) with the above-described (17a).

(22R,23R,24S)-22,23-*Dihydroxy*-B-*homo*-7-*oxa*-5α-*stigmastane*-3,6-*dione* (22).—A mixture of the 3α-ol (20b) and the 3β-ol (17a) (27 mg, 0.0538 mmol) in dichloromethane (3 ml) was treated with pyridinium chlorochromate (20 mg, 0.0930 mmol) at room temperature for 3 h. To the reaction mixture, ether (50 ml) was added. Filtration through a Florisil column and removal of the solvent gave (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy-B-homo-7-oxa-5α-stigmastane-3,6-dione (24 mg, 89%), m.p. 243—244 °C (from ethyl acetate–hexane); δ ($CDCl_3$) 0.72 (3 H, s, 18-H_3), 1.10 (3 H, s, 19-H_3), 1.35 (6 H, s, acetonide), 2.70—3.40 (2 H, m), 3.76 (2 H, bs, $W_{\frac{1}{2}}$ 4 Hz, 22- and 23-H), and 4.06 (2 H, m, 7-H_2); high resolution mass spectrum, *m/z* 516.3821 (M^+; calc. for $C_{32}H_{52}O_5$, *m/z* 516.3817). This product was dissolved in THF (2 ml), 70% aqueous perchloric acid (0.1 ml) was added and the mixture, was stirred at room temperature for 1 h. The usual work-up (ethyl acetate extraction) provided the *diol* (22) (21 mg, 98%), m.p. 270—272 °C (from ethyl acetate), δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 3.58 (1 H, d, *J* 9 Hz, 22-H), 3.72 (1 H, d, *J* 23-H), and 4.06 (2 H, m, 7-H_2) (Found: C, 73.2; H, 10.2. $C_{29}H_{48}O_5$ requires C, 73.07; H, 10.15%).

(22R,23R,24S)-3α,22,23-*Trihydroxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (21).—The acetonide (20a) (35 mg, 0.064 mmol) was treated with 70% aqueous acetic acid (5 ml) under reflux for 5 h. Removal of the solvent under reduced pressure gave a crude product, which was saponified with 5% KOH–MeOH (5 ml) under reflux for 1 h and then cooled to room temperature. 6M-HCl (5 ml) was added and the mixture was stirred at room temperature for 1 h. The usual work-up (ethyl acetate extraction) provided 2-*deoxy*-28-*homobrassinolide* (21) (29 mg, 95%), m.p. 216—217 °C (from ethyl acetate), δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 3.10 (1 H, dd, *J* 12 and 4 Hz, 5α-H), 3.58 (1 H, d, *J* 9 Hz, 22-H), 3.72 (1 H, d, *J* 9 Hz, 23-H), 4.10 (2 H, m, 7-H_2), and 4.18 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H) (Found: C, 72.7; H, 10.55. $C_{29}H_{50}O_5$ requires C, 72.76; H, 10.53%).

(22R,23R,24S)-2α,3α,22,23-*Tetrahydroxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (1b).—The 2-ene (18) (20 mg, 0.040 mmol) was hydroxylated with osmium tetraoxide, as described for

(2b), to give the 2α,3α-diol (21 mg). This was deprotected with 80% aqueous acetic acid (5 ml) under reflux for 4 h. Removal of the solvent under reduced pressure and chromatography on silica gel (15 g) eluting with ethyl acetate provided the (22*R*,23*R*,24*S*)-28-homobrassinolide (1b) (16 mg, 90%), m.p. 269—271 °C (lit.,[10] m.p. 268—271 °C) (from ethyl acetate), $\nu_{max.}$ (KBr) 3 450 (s), 2 972 (s), 2 948 (s), 2 880 (s), 2 850 (m), 1 732 (m), 1 715 (sh), 1 701 (s), 1 650 (w), 1 470 (m), 1 460 (m), 1 445 (w), 1 409 (m), 1 388 (m), 1 333 (m), 1 320 (sh), 1 300 (w), 1 282 (m), 1 260 (w), 1 230 (m), 1 190 (m), 1 147 (m), 1 130 (m), 1 080 (sh), 1 067 (s), 1 040 (sh), 1 030 (m), 1 018 (sh), 990 (m), and 940 cm^{-1} (w); field desorption mass spectrum, *m/z* 495 (M + 1), 477, 379, 349, 145, and 115 (Found: C, 70.35; H, 10.3. Calc. for $C_{29}H_{50}O_6$: C, 70.41; H, 10.19%).

(22R,23R,24S)-3α,4α,22,23-*Tetrahydroxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (23).—The 3-ene (19) (30 mg, 0.060 mmol) was hydroxylated with osmium tetraoxide, as described for (2b), to provide the 3α,4α-diol (30 mg), which was treated with 80% aqueous acetic acid under reflux for 4 h. Removal of the solvent under reduced pressure and chromatography on silica gel (20 g), eluting with benzene–ethyl acetate (1 : 5), provided the *tetraol* (23) (26 mg, 88%), m.p. 268—269 °C (from methanol), δ (C_5D_5N–$CDCl_3$, 1 : 1; 200 MHz) 0.72 (3 H, s, 18-H_3), 0.95 (3 H, s, 19-H_3), 3.30 (1 H, d, *J* 10 Hz, 5α-H), 3.78 (1 H, d, *J* 8 Hz, 22-H), 3.92 (1 H, d, *J* 8 Hz, 23-H), 4.08 (1 H, m, 7-H_2), 4.12 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H), 4.42 (1 H, dd, *J* 10 and 3 Hz, 4β-H) (Found: C, 70.4; H, 10.3. $C_{29}H_{50}O_6$ requires C, 70.41; H, 10.19%).

(22R,23R,24S)-22,23-*Dihydroxy*-B-*homo*-7-*oxa*-5α-*stigmastan*-6-*one* (24).—The 2-ene (18) (10 mg, 0.020 mmol) in ethyl acetate (3 ml) was stirred with 5% Pd–C (5 mg) under hydrogen for 5 h. Filtration and removal of the solvent gave (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy-B-homo-7-oxa-5α-stigmastan-6-one (10 mg), m.p. 238—239 °C (from methanol), δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 2.68 (1 H, dd, *J* 10 and 6 Hz, 5α-H), 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 4 Hz, 22- and 23-H), and 4.02 (2 H, m, 7-H_2); high resolution mass spectrum, *m/z* 502.4014 (M^+; calc. for $C_{32}H_{54}O_4$: *m/z* 502.4025). This was treated with 70% aqueous acetic acid (5 ml) under reflux for 4 h. Removal of the solvent gave the 2,3-*dideoxy*-28-*homobrassinolide* (24) (9.5 mg), as an amorphous solid; δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 2.68 (1 H, dd, *J* 10 and 6 Hz, 5α-H), 3.58 (1 H, d, *J* 9 Hz, 22-H), 3.72 (1 H, d, *J* 9 Hz, 23-H), and 4.02 (2 H, m, 7-H_2); high resolution mass spectrum, *m/z* 462.3706 (M^+, calc. for $C_{29}H_{50}O_4$: *m/z* 462.3711).

(22R,23R,24S)-3β,22,23-*Trihydroxy*-B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (15b).—The triacetate (15a) (155 mg, 0.257 mmol) was treated with 5% KOH–MeOH (20 ml) under reflux for 1 h. To the cooled reaction mixture, 6M HCl (20 ml) was added and the mixture was stirred at room temperature for 1 h. The usual work-up (ethyl acetate extraction) gave the *triol* (15b) (120 mg, 98%), m.p. 255—256 °C (from ethyl acetate); δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 3.45 (1 H, m, 3α-H), 3.58 (1 H, d, *J* 9 Hz, 22-H), 3.72 (1 H, d, *J* 9 Hz, 23-H), and 4.21 (1 H, dd, *J* 12 and 5 Hz, 5α-H) (Found: C, 72.75; H, 10.55. $C_{29}H_{50}O_5$ requires C, 72.76; H, 10.53%).

(22R,23R,24S)-3β-*Hydroxy*-22,23-*isopropylidenedioxy*-B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (25a).—The triol (15b) (94 mg, 0.197 mmol) was converted into (25a) as described for (16a)—(17a). The product (25a) (102 mg) had δ ($CDCl_3$) 0.68 (3 H, s, 18-H_3), 1.33 (6 H, s, acetonide), 2.40 (2 H, m, 7-H_2), 3.45 (1 H, m, 3α-H), 3.74 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), and 4.21 (1 H, dd, *J* 12 and 5 Hz, 5α-H).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-3β-*methylsulphonyloxy*-B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (25b).—The 3β-ol (25a) (102 mg) was mesylated as described for (17b) to give the 3β-methanesulphonate (25b) (117 mg); δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 1.34 (6 H, s, acetonide), 2.43 (2 H, m, 7-H_2), 3.00 (3 H, s, mesyl), 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), and 4.10—4.70 (2 H, m, 3α- and 5-H).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-B-*homo*-6-*oxa*-5α-*stigmast*-3-*en*-7-*one* (27).—The methanesulphonate (25b) (117 mg) was treated with lithium carbonate as described for (17b) to give the crude product (89 mg). This was purified by preparative t.l.c. (benzene–ethyl acetate, 10 : 1, developed twice) to provide the 3-*ene* (27) [14 mg, 14% from (15a)], m.p. 228—229 °C (from methanol), R_F 0.68, δ ($CDCl_3$) 0.71 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 2.50 (2 H, m, 7-H_2), 3.76 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 4.75 (1 H, m, $W_{\frac{1}{2}}$ 6 Hz, 5α-H), and 5.30—6.00 (2 H, m, 3- and 4-H); high resolution mass spectrum, *m/z* 500.3870 (M^+, $C_{32}H_{52}O_4$ requires *m/z* 500.3868).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-B-*homo*-6-*oxa*-5α-*stigmast*-2-*en*-7-*one* (26).—The 2-*ene* (26) [26 mg, 26% from (15a)], m.p. 195—197 °C (from methanol), R_F 0.63; δ ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 1.36 (6 H, s, acetonide), 2.42 (2 H, m, 7-H_2), 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 4.43 (1 H, dd, *J* 9 and 8 Hz, 5α-H), and 5.50 (2 H, m, 2- and 3-H); high resolution mass spectrum, *m/z* 500.3873 (M^+, $C_{32}H_{52}O_4$ requires *m/z* 500.3868).

(22R,23R,24S)-3α-*Formyloxy*-22,23-*isopropylidenedioxy*-B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (28a).—The 3α-*formate* (28a) [14 mg, 13% from (15a)], an oil R_F 0.32, δ ($CDCl_3$) 0.70 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 2.46 (2 H, m, 7-H_2), 3.76 (2 H, bs, $W_{\frac{1}{2}}$ 3 Hz, 22- and 23-H), 4.48 (1 H, dd, *J* 12 and 6 Hz, 5α-H), 5.27 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H), and 8.01 (1 H, s, formyl); high resolution mass spectrum, *m/z* 500.3865 (M^+ − CH_2O, $C_{32}H_{52}O_4$ requires *m/z* 500.3868).

(22R,23R,24S)-3α-*Hydroxy*-22,23-*isopropylidenedioxy*-B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (28b).—The most polar band (R_F 0.02) was again purified by preparative t.l.c. (chloroform–methanol, 10 : 1, developed once) to provide the 3α-*ol* (28b) [12 mg, 11% from (15a)], oil, R_F 0.52, δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 1.35 (6 H, s, acetonide), 2.49 (2 H, m, 7-H_2), 3.75 (2 H, bs, $W_{\frac{1}{2}}$ 4 Hz, 22- and 23-H), 4.18 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 3β-H), and 4.60 (1 H, dd, *J* 12 and 6 Hz, 5α-H); high resolution mass spectrum, *m/z* 518.3975 (M^+, $C_{32}H_{54}O_5$ requires *m/z* 518.3973).

(22R,23R,24S)-3α,22,23-*Trihydroxy*-B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (29).—The 3α-formate (28a) (14 mg) and the 3α-ol (28b) (12 mg) were combined and this mixture was converted, as described for (21), into the *triol* (29) (21 mg, 88%), m.p. 239—240 °C (from ethyl acetate); δ ($CDCl_3$) 0.69 (3 H, s, 18-H_3), 2.50 (2 H, m, 7-H_2), 3.58 (1 H, d, *J* 9 Hz, 22-H), 3.72 (1 H, d, *J* 9 Hz, 23-H), 4.18 (1 H, m, $W_{\frac{1}{2}}$ 7 Hz, 3β-H), and 4.60 (1 H, dd, *J* 12 and 6 Hz, 5α-H) (Found: C, 72.8; H, 10.55. $C_{29}H_{50}O_5$ requires C, 72.76; H, 10.53%).

(22R,23R,24S)-2α,3α,22,23-*Tetrahydroxy*-B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (31).—The 2-ene (26) (24 mg, 0.048 mmol) was converted, as described for (2b) into the *tetrahydroxy*-6-*oxalactone* (31) (22 mg, 92%), m.p. 277—279 °C (from methanol), δ (C_5D_5N–$CDCl_3$, 1 : 2; 200 MHz) 0.71 (3 H, s, 18-H_3), 0.95 (3 H, s, 19-H_3), 2.50 (2 H, m, 7-H_2), 3.67 (1 H, d, *J* 8 Hz, 22-H), 3.83 (1 H, d, *J* 8 Hz, 23-H), 4.01 (1 H, m, $W_{\frac{1}{2}}$ 20 Hz, 2β-H), 4.12 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H), and

4.73 (1 H, dd, *J* 12 and 6 Hz, 5α-H) (Found: C, 70.4; H, 10.2. $C_{29}H_{50}O_6$ requires C, 70.41; H, 10.19%).

(22R,23R,24S)-3α,4α,22,23-*Tetrahydroxy*-B-*homo*-6-*oxa*-5α-*stigmastan*-7-*one* (30).—The 3-ene (27) (13 mg, 0.026 mmol) was converted, as described for (2b), into the *tetrahydroxy*-6-*oxalactone* (30) (11.4 mg, 89%), m.p. 254—255 °C (from methanol); δ (C_5D_5N–$CDCl_3$, 1 : 1; 200 MHz) 0.72 (3 H, s, 18-H_3), 0.94 (3 H, s, 19-H_3), 2.49 (2 H, m, 7-H_2), 3.77 (1 H, d, *J* 8 Hz, 22-H), 3.91 (2 H, d, *J* 8 Hz, 23- and 4β-H), 4.27 (1 H, m, $W_{\frac{1}{2}}$ 8 Hz, 3β-H), and 4.60 (1 H, d, *J* 10 Hz, 5α-H) (Found: C, 70.45; H, 10.2. $C_{29}H_{50}O_6$ requires C, 70.41; H, 10.19%).

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