# Improved synthesis of brassinolide

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**Brassinolide has been synthesized from stigmasterol in an overall yield of 7%. The key step in the synthesis is aldol condensation of 2α,3α-isopropylidenedioxy-6-oxo-23,24-dinor-5α-cholan-22-al with 3-isopropylbut-2-enolide carried out at −78 °C, which gives a product with 22*R*,23*R* stereochemistry in high yield. Catalytic hydrogenation of this product is highly stereoselective leading to the desired 24*S* stereochemistry in an intermediate which is readily transformed into brassinolide.**

## Introduction

The elucidation of structure of the steroidal plant-growth regulator brassinolide **1** was first reported in 1979.[1] This achievement was the result of many years' effort by John W. Mitchell and other scientists at the USDA laboratories in Beltsville, Philadelphia and Peoria, to identify the substance in pollen of the rape plant *Brassica napus* responsible for accelerated growth of internode sections of bean plants. The so-called bean second-internode assay had been developed for detecting gibberellins.[2] Brassinolide was extremely active and at a concentration of 0.1 μg per plant it caused the unique effect of splitting the internode.

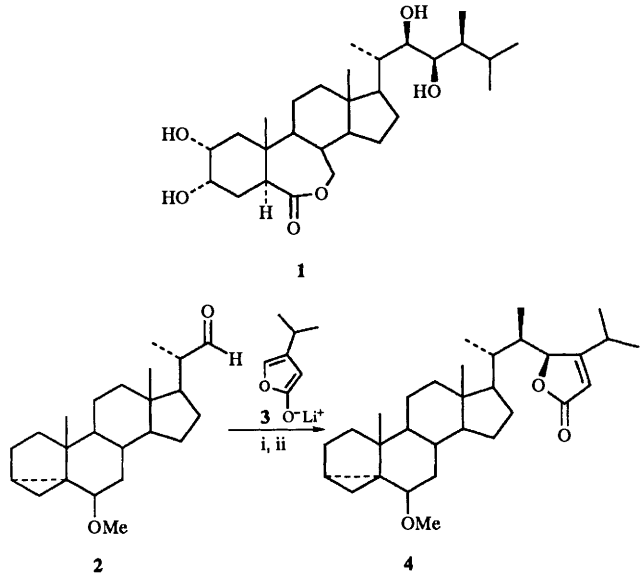
In the past fifteen years over sixty analogues of brassinolide (brassinosteroids) have been detected in a wide variety of plants.[3] Brassinolide remains the most biologically active of brassinosteroids. It acts at very low concentrations ($10^{-9}$ mol $dm^{-3}$) and shows strong synergistic interactions with auxin, and additive interactions with gibberellins, in many test systems.[2]

Recently, molecular and genetic evidence for a possible independent mechanism for auxin and brassinolide action has been found.[4] Nanomolar concentrations of brassinolide altered the abundance of specific mRNA transcripts in elongating soybean epicotyls and *Arabidopsis thaliana* stems.[5] This suggests that brassinolide, like other plant and animal hormones, acts in part by regulating gene expression.

Some preliminary tests of brassinosteroids by the USDA Beltsville group have suggested that brassinosteroids can be used for increasing food production.[2] This has led to world-wide studies on application of brassinosteroids in agriculture. At the present time there appears to be some controversy as to the efficacy of brassinosteroids in field tests. Thus while the results obtained in field trials in China have been reported to be clearly positive,[6] results from field trials in Japan have been disappointing.[7]

The brassinosteroid most available for testing is 24-epibrassinolide[6] which has been synthesized by many groups.[8] It can be made readily from ergosterol, an abundant sterol.[9] 28-Homobrassinolide is likewise now readily prepared from abundant stigmasterol.[10] Many syntheses of brassinolide have been reported.[11] The majority of these employ a C-22 aldehyde, derived from stigmasterol, for construction of the sidechain. The aldehyde is allowed to react with a carbanion containing a double bond (or potential double bond). Stereoselective epoxidation of the double bond followed by stereospecific oxirane ring opening leads to the brassinolide sidechain.

In a communication in 1984 we reported a synthesis of brassinolide, the key step of which was condensation of a C-22 aldehyde **2**, with the anion **3** derived from 3-isopropylbut-2-enolide[12] (Scheme 1). The product **4** was then converted into brassinolide by manipulations of the functional groups on the sidechain and on the tetracyclic nucleus.[13] In view of the potential importance of brassinolide in agriculture we have been encouraged to improve our synthetic method. This paper describes in detail a much improved synthesis which has been used to produce several grams of pure brassinolide.




**Scheme 1** *Reagents and conditions:* i, THF, −78 °C, 5 h; ii, dil. HCl, −78 °C

## Results and discussion

The new method also involves a C-22 aldehyde, but which possesses 2α,3α-dihydroxy-6-keto groups, so that, after aldol condensation, only one step is required to transform the tetracyclic nucleus into that of brassinolide (Scheme 2). Moreover, the aldehyde **10** can be readily prepared from stigmasterol **5** in an overall yield of 42%. Stigmasterol was first converted into the *i*-sterol *via* the mesyl derivative. Oxidation of the *i*-sterol gave the corresponding ketone **6** (92% yield). Introduction of the $\Delta^2$ double bond was best effected by the procedure of Takatsuto.[14] Treatment of compound **6** with lithium bromide in the presence of pyridinium chloride in refluxing dimethylacetamide (DMA) for 4 h afforded an 80% yield of the desired $\Delta^2$ compound **7** after recrystallization. Without lithium bromide only a small amount of product was observed after 5 h. Presumably, this is a two-step process involving acid-catalysed bromide opening of the cyclopropane ring to give the 3β-bromo-6-ketone, which on dehydrobromination affords the desired $\Delta^2$-ene.

Dihydroxylation of the dienone **7** with $OsO_4$–*N*-methylmorpholine *N*-oxide (NMO) for 12 h at room temperature gave the 2α,3α-diol in 65% yield. A little 2β,3β-diol (8%) and 2α,3α,22*S*,23*S*-tetraol (20%) were also obtained. Longer reaction times resulted in higher yields of the tetraol.

Conversion of the 2α,3α-diol into the acetonide **8** [2,2-dimethoxypropane, camphor-10-sulfonic acid (CSA)] followed by ozonolysis at −78 °C in methanol–dichloromethane and reductive work up (dimethyl sulfide) gave the desired aldehyde **10** but in only fair yield. Different conditions were tried and the best were found to be ozonolysis in dichloromethane–pyridine (94:6) and reductive work-up with zinc dust. However, the yield of aldehyde was only 48%, the other main product being the corresponding carboxylic acid (30%).

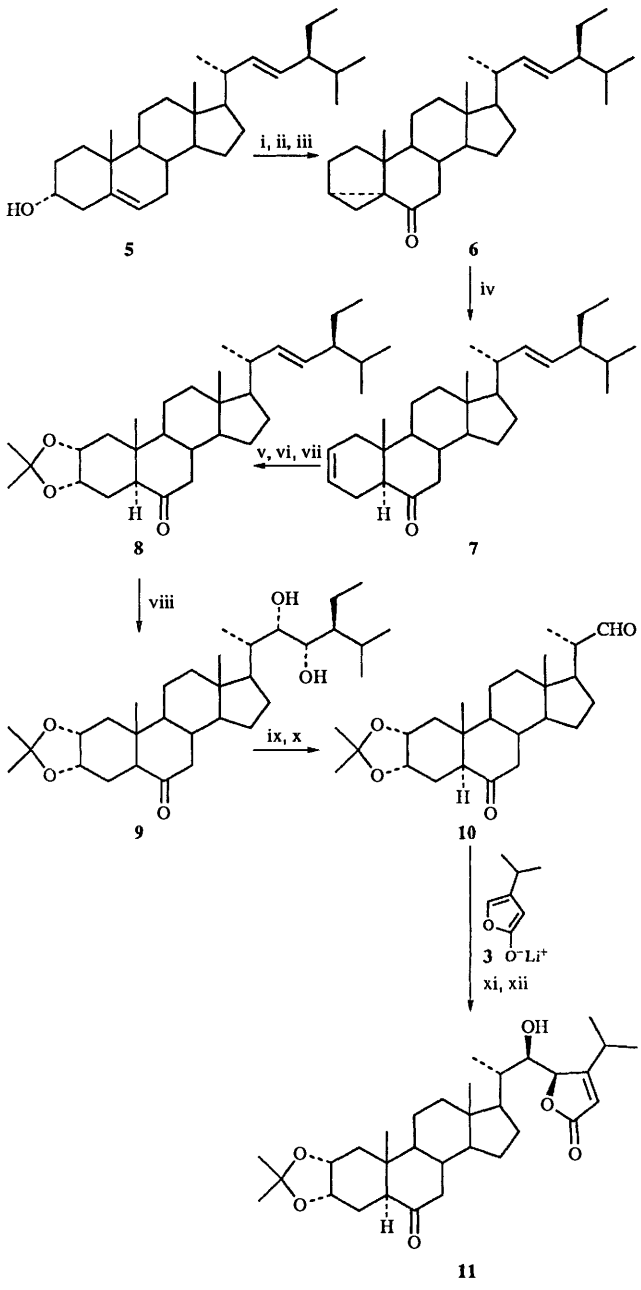
An alternative route to the aldehyde **10** was therefore employed. It involved dihydroxylation of the $\Delta^{22}$ double bond followed by periodic acid cleavage. Thus the acetonide **8** was further hydroxylated to the 22*S*,23*S* diol with $OsO_4$–NMO. Initially, $K_2CO_3$ and $MeSO_2NH_2$ were also added to the reaction mixture as recommended adjuvants in catalytic asymmetric dihydroxylation.[15] However, very slow rates were observed. Reaction could be accelerated by warming of the reaction mixture to 40 °C for 7 days, leading to 85% completion. Without $K_2CO_3$ extensive acetonide deprotection occurred. When $NaHCO_3$ was used instead, complete reaction was accomplished in 7 days. The product (98% yield) was obtained as a mixture of 22*S*,23*S*-diol **9** and 22*R*,23*R* diol in a 9:1 ratio.

Attempts to cleave diol **9** with $NaIO_4$ in aq. acetone failed. On the other hand, cleavage with $H_5IO_6$ in tetrahydrofuran (THF) occurred rapidly though there was also extensive loss of the 2,3-acetonide protecting group followed by further reaction of the 2,3-diol. These undesired reactions could be prevented by adding a small amount of pyridine. Under these conditions the diol **9** was completely cleaved in 24 h to give the desired aldehyde **10** in 82% yield from diol **9**.

Having secured aldehyde **10**, we next carried out studies on aldol condensation with the anion from 3-isopropylbutenolide formed with lithium diisopropylamide (LDA). The best yields of the desired 22*R*,23*R* condensation product were obtained by preparing the anion at −40 °C then cooling the solution to −78 °C before addition of the aldehyde (in the ratio 1 mole equivalent aldehyde to 6 mole equivalents anion). The mixture was maintained below −70 °C for 14 h, to ensure complete reaction, and quenched with aq. $NH_4Cl$ at that temperature. Work-up gave an 80% yield of product **11**. The ketone was unreactive in the aldol reaction so it was not necessary to protect it. In fact the acetal (protected ketone) was unstable and the protecting group tended to fall off during work-up, leading to mixtures. The aldol reaction was highly stereoselective and only a minor amount of the 22*R*,23*S* product (9%) was isolated.

Similar high stereoselectivity was obtained previously in a reaction with the related aldehyde **2** and the result has been rationalized by applying the Cram or Felkin-Anh rules for stereochemistry at C-22.[12] The stereochemistry at C-23 could be explained as resulting from kinetic control in the aldol reaction. When the reaction was carried out at higher temperature the major product was the 22*R*,23*S* isomer (thermodynamic product). The structure of the kinetic product **11** was confirmed by X-ray crystallographic analysis of the corresponding 2α,3α-dihydroxy-6-ketone (Fig. 1).

In contrast to the aldol condensation described above, a similar reaction with aldehyde **10** and 6 mole equivalents of the anion from 2,3-dimethylbutenolide at −78 °C was complete within 5 min. It was quenched at that temperature with aq. $NH_4Cl$. The 22*R*,23*R* isomer and 22*R*,23*S* isomer were isolated in yields of 57 and 32%, respectively. This difference in rate of reaction and ratio of products can be attributed to steric factors (*i.e.*, isopropyl *vs.* methyl). The use of 2,3-dimethylbutenolide




**Scheme 2** *Reagents and conditions:* i, $MeSO_2Cl$, Py; ii, $KHCO_3$, aq. acetone, reflux, 6 h; iii, $CrO_3$, $H_2SO_4$, acetone; iv, LiBr, Py·HCl, DMA 160 °C, 4 h; v, $OsO_4$ in Bu$^t$OH, aq. acetone, NMO; vi, aq. $NaHSO_3$; vii, $Me_2C(OMe)_2$, $H^+$, $CH_2Cl_2$–MeCN, room temp., 1 h; viii, $OsO_4$ in Bu$^t$OH, NMO, aq. THF, 7 d, 40 °C; ix, dry THF, Py, $NaHCO_3$, $H_5IO_6$, room temp., 24 h; x, aq. $NaHSO_3$; xi, THF, −78 °C, 14 h; xii, aq. $NH_4Cl$, −78 °C

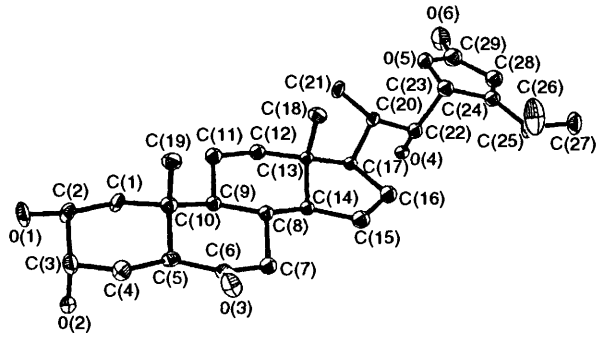
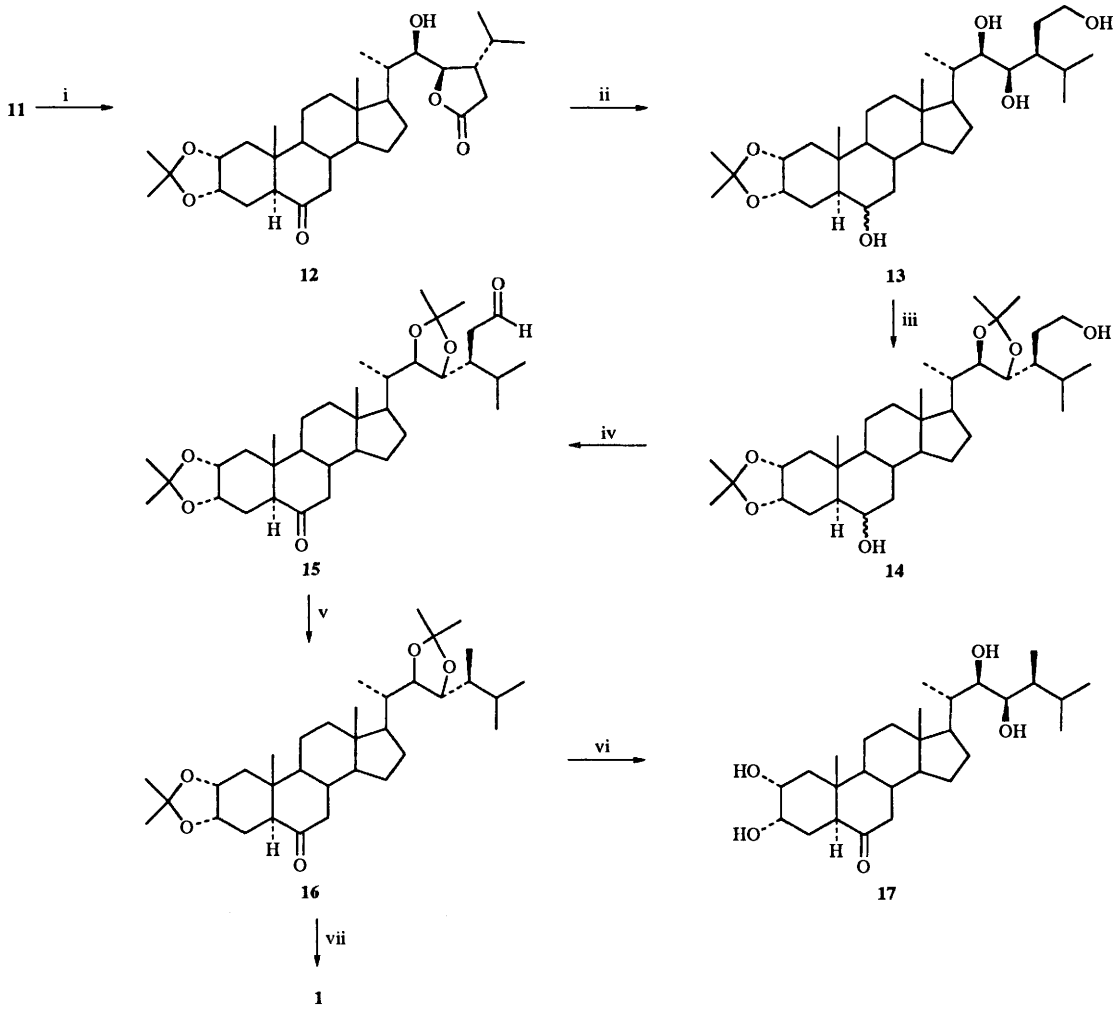



**Fig. 1** ORTEP view of the intermediate (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-6-oxo-5α-sitost-24(28)-en-29-oic acid γ-lactone




**Scheme 3** *Reagents and conditions:* i, $H_2$/Pd/C, THF–EtOH–Py, room temp., 50 psi, 14 h; ii, LAH, THF, Ar, reflux, 12 h; iii, acetone, CSA, room temp., $N_2$, 24 h; iv, $CrO_3$–Py, $CH_2Cl_2$, room temp., 48 h; v, $[(C_6H_5)_3P]_3RhCl$, $C_6H_6$, Ar, reflux, 5 h; vi, TFA, MeOH; vii, TFAA, $H_2O_2$ (30%), $CHCl_3$, room temp., 2 h

to construct the sidechain of brassinolide thus offered no advantage over 3-isopropylbutenolide.[12]

The next step in the synthesis was hydrogenation of the $\Delta^{24,28}$ double bond (Scheme 3). This was performed satisfactorily using 10% Pd/C in THF containing a small amount of pyridine in a Parr hydrogenation apparatus ($H_2$ pressure, 50 psi) for 18 h. A quantitative yield of a mixture of 24*R* isomer and 24*S* isomer (**12**) in a 1:5 ratio was obtained. (The configurations were established by the fact that the major isomer was converted into brassinolide.) The observed stereochemistry in favour of the desired 24*S* isomer may be a result of the directing influence that the hydroxy group at C-22 has on the addition of hydrogen.

Having completed construction of all stereocentres on the sidechain with high stereoselectivity, the remaining steps in the synthesis were relatively straightforward. Reduction of lactone **12** with $LiAlH_4$ afforded a quantitative yield of the tetraol **13** (epimeric mixture 6β:6α hydroxy 6:1). Protection of the C-22, C-23 hydroxy groups as the acetonide was best accomplished by treating tetraol **13** with anhydrous acetone and a catalytic amount of CSA under nitrogen at room temperature. With these conditions no formation of hemiacetal with the C-29 hydroxy group was observed.[12] Oxidation of the bisacetonide **14** with Collins reagent afforded a 91% yield of the keto aldehyde **15**. Other oxidants, *e.g.*, pyridinium chlorochromate, resulted in somewhat lower yields because of loss of the acetonide. Keto aldehyde **15** was smoothly decarbonylated with tris(triphenylphosphine)rhodium(I) chloride (Wilkinson's catalyst) in refluxing benzene under argon for 5 h. A good yield (~72%) of castasterone bisacetonide **16** was obtained.

Castasterone bisacetonide **16** could be converted directly into brassinolide by adding it to trifluoroacetic acid (TFA) followed by treatment with excess of trifluoroperoxyacetic acid for 2 h. As part of the work-up, the reaction mixture was treated with methanolic $K_2CO_3$ followed by acidification, in order to hydrolyse any trifluoroacetate esters. The Baeyer–Villiger oxidation afforded a mixture of 7-oxalactone and 6-oxalactone in a 9:1 ratio from which pure brassinolide could be obtained by fractional crystallization from methanol in 62% yield. The unusual regioselectivity in this reaction has been observed by several investigators.[16] The structure of brassinolide was confirmed by all spectral data and also an X-ray crystallographic analysis. The overall yield of brassinolide starting from stigmasterol was 7%, which is higher than previously reported syntheses. Biological activity of brassinolide was greater in the soybean epicotyl elongation assay than that of its naturally occurring analogues and has been reported elsewhere.[10]

## Experimental

Mps were determined on a Kofler hot-stage microscope and are uncorrected. High-resolution mass spectra were obtained at the University of Minnesota, Mass Spectrometry Service Laboratory. $^1H$ NMR spectra (360 MHz) were taken in $CDCl_3$ with $CHCl_3$ (resonance at $\delta$ 7.260) as well as tetramethylsilane as internal standards. For the $^{13}C$ NMR spectra, chemical shifts were calculated relative to the $CDCl_3$ resonance at $\delta_C$ 77.0. *J*-Values are given in Hz. X-Ray structures were obtained at the UCSD Chemistry Department X-Ray Crystallography Facility by Dr Peter Gantzel.

Unless otherwise indicated, all solvents and reagents used were of commercial grade. Dry THF was freshly obtained by heating the liquid at reflux, under nitrogen, in a recirculation still over potassium. Dichloromethane was distilled from calcium hydride. Pyridine was purified by distillation and stored over KOH pellets. Diisopropylamine was purified by distillation from $CaH_2$ and stored over KOH pellets. Benzene was distilled from sodium.

Mixed-solvent flash chromatography was performed by first equilibrating the silica gel (Fisher Scientific, 230–425 mesh) in the less polar solvent, and loading the sample in a minimum amount of the mixed solvent. Products were then eluted using a solvent gradient with increasing percentage of the more polar solvent.

Reactions were monitored by TLC on Whatman aluminium-backing silica gel plates. Spots were visualized with vanillin reagent made by dissolution of vanillin (3 g) in ethanol (100 $cm^3$) and conc. sulfuric acid (3 $cm^3$). Spots were developed by heating.

**(22*E*,24*S*)-5α-Stigmasta-2,22-dien-6-one 7**

A mixture consisting of the ketone **6**[10] (30.0 g, 0.073 mol), LiBr (3.17 g, 0.036 mol), pyridine hydrochloride (4.16 g, 0.036 mol) and DMA (240 $cm^3$) was heated at 160 °C for 4 h. The first crop of product crystallized upon cooling (60%). The cold suspension was filtered, and the crystals were washed with cold MeOH. After removal of MeOH, the filtrate was transferred to a separatory funnel and extracted with hexanes (4 × 200 $cm^3$). The hexane extracts were washed successively with saturated aq. $(NH_4)_2SO_4$ (2 × 400 $cm^3$) and brine (400 $cm^3$), and dried ($MgSO_4$). In order to remove the coloured impurity, the dried solution was filtered through a short column of silica (30 g) which was flushed with hexane. The solvent was evaporated off. The second crop was obtained as prisms from a minimum amount of hot EtOH. The combined yield of the product was 24.0 g (80%); mp 119–120 °C (lit.,[17] 119–120 °C); TLC: hexane–acetone (95:5) $R_F$ = 0.49; $\delta_H$ 0.69 (3 H, s, 18-$H_3$), 0.71 (3 H, s, 19-$H_3$), 0.78–0.86 (9 H, m, 26-, 27-and 29-$H_3$), 1.02 (3 H, d, *J* 6.6, 21-$H_3$), 2.35 (2 H, d, *J* 4.08, 7-$H_2$), 2.35 (1 H, dd, *J* 4.2 and 3.96, 5α-H), 5.02 (1 H, dd, *J* 15.3 and 8.4, 22-H), 5.15 (1 H, dd, *J* 15.3 and 8.4, 23-H), 5.53–5.61 (1 H, m, 2-H) and 5.64–5.73 (1 H, m, 3-H); $\delta_C$ 212.0, 137.9, 129.5, 124.9, 124.5, 56.8, 55.9, 53.8, 53.4, 51.2(2), 46.7, 42.7, 40.4, 39.4(2), 37.7, 31.8, 28.7, 25.4, 24.0, 21.7, 21.2, 21.1(2), 18.9, 13.5, 12.2 and 12.1.

**(22*E*,24*S*)-2α,3α-Dihydroxy-5α-stigmast-22-en-6-one**

Dienone **7** (5.00 g, 0.012 mol) was dissolved in a mixture of acetone–water (9:1) (150 $cm^3$). $OsO_4$ (3.8 $cm^3$, 2.5% in Bu$^t$OH) was added followed by NMO (3.51 g, 0.03 mol, 2.5 mol equiv.). The mixture was stirred for 12 h, after which TLC (hexane–Pr$^i$OH, 4:1) showed almost complete disappearance of the starting material, and appearance of three other compounds. Aq. $NaHSO_3$ [3.0 g, 0.029 mol, 2.4 mol equiv. in water (10 $cm^3$)] was added, and the mixture was stirred for another 5 h. The solvent was evaporated off and the residue was dissolved in $CH_2Cl_2$ (300 $cm^3$) which was washed successively with 5% aq. $NaHSO_3$ (100 $cm^3$), saturated aq. $NaHCO_3$ (100 $cm^3$) and brine (100 $cm^3$), dried ($MgSO_4$) and filtered. The filtrate was evaporated and the desired product was recrystallized from hot DMA (50 $cm^3$). The crystals were filtered, and washed with cold methanol. The yield of 2α,3α-diol was 3.52 g (65%), mp 238–240 °C (plates) (lit.,[11e] 235–238 °C).

In a separate experiment, dihydroxylation of dienone **7** (5.0 g, 0.012 mol) afforded the same mixture of products which was separated by chromatography ($CH_2Cl_2$–EtOAc gradient). The first product to be eluted was (22*E*,24*S*)-2β,3β-dihydroxystigmast-22-en-6-one (0.43 g, 8%); mp 213–215 °C (plates from DMA); TLC: $CH_2Cl_2$–EtOAc (7:3) $R_F$ = 0.17; $\delta_H$ 0.68 (3 H, s, 18-$H_3$), 0.78–0.85 (9 H, m, 26-, 27-, 29-$H_3$), 0.98 (3 H, s, 19-$H_3$), 1.02 (3 H, d, *J* 6.5, 21-$H_3$), 2.13 (1 H, dd, *J* 14.5 and 3.0, 1β-H), 2.21 (1 H, dd, *J* 12.0 and 3.0, 5α-H), 2.31 (1 H, dd, *J* 13.5 and 5.0, 7β-H), 3.63 (1 H, m, 3-H), 4.03 (1 H, d, *J* 2.7, 2-H), 5.02 (1 H, dd, *J* 15.5 and 8.5, 22-H) and 5.12 (1 H, dd, *J* 15.5 and 8.5, 23-H); $\delta_C$ 210.8, 137.9, 129.6, 71.8, 69.1, 57.1, 56.8, 55.9, 54.7, 51.2, 46.5, 42.9, 42.4, 40.6, 40.4, 39.4, 37.2, 31.8, 28.7, 25.4, 24.2, 24.0, 21.5, 21.1(2), 18.9, 15.2 and 12.2(2); $\nu_{max}$(KBr)/$cm^{-1}$ 3380, 2955, 2870 and 1705.

Further elution with the solvent gradient gave the more polar (22*E*,24*S*)-2α,3α-dihydroxy-5α-stigmast-22-en-6-one (3.90 g, 72%); mp 238–240 °C (from DMA) [lit.,[11e] 235–238 °C (from 99% EtOH)]; TLC: $CH_2Cl_2$–EtOAc (7:3) $R_F$ = 0.14; $\delta_H$ 0.67 (3 H, s, 18-$H_3$), 0.75 (3 H, s, 19-$H_3$), 0.77–0.85 (9 H, m, 26-, 27-, 29-$H_3$), 1.01 (3 H, *J* 6.6, 21-$H_3$), 2.3 (1 H, dd, *J* 13.2 and 4.5, 7β-H), 2.67 (1 H, dd, *J* 9.3 and 3.0, 7α-H), 3.75 (1 H, m, 2β-H), 4.05 (1 H, dd, *J* 2.4, 3β-H), 5.02 (1 H, dd, *J* 15.3 and 8.4, 22-H) and 5.14 (1 H, dd, *J* 15.3 and 8.4, 23-H); $\delta_C$ 212.1, 137.9, 129.6, 68.4, 68.3, 56.8, 55.8, 53.8(2), 51.2, 50.7, 46.7, 42.8, 42.6, 40.4, 40.2, 39.3, 37.7, 31.9, 28.7, 26.3(2), 25.4, 24.0, 21.2, 21.1, 18.9, 13.6 and 12.2; $\nu_{max}$(KBr)/$cm^{-1}$ 3396, 2942, 2867 and 1708.

The last product eluted with the solvent gradient corresponded to (22*S*,23*S*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-stigmastan-6-one (0.325 g, 6%); mp 214–215 °C (from MeOH) [lit.,[11e] 206–208 °C]; TLC: $CH_2Cl_2$–EtOAc (7:3) $R_F$ = 0.07; $\delta_H$ 0.69 (3 H, s, 18-$H_3$), 0.75 (3 H, s, 19-$H_3$), 0.88 (3 H, d, *J* 6.9), 0.92–1.04 (6 H, m, 2 × Me), 2.22 (1 H, d, *J* 6.3), 2.3 (1 H, dd, *J* 13.0 and 4.3), 2.68 (1 H, dd, *J* 12.5 and 3.0, 5α-H), 3.59 (2 H, m, 22-and 23-H), 3.75 (1 H, m, 2-H) and 4.03 (1 H, br s, 3-H); $\delta_C$ 212.0, 72.1, 70.6, 68.3, 68.2, 56.3, 53.6, 52.6, 50.7, 49.6, 46.7, 43.5, 42.5, 42.3, 40.2, 39.3, 37.6, 27.8, 26.9, 26.3, 24.2, 21.7, 21.2, 18.5, 17.7, 14.5, 14.1, 13.5 and 11.9; $\nu$(KBr)/$cm^{-1}$ 3412, 2951, 2872 and 1702.

**(22*E*,24*S*)-2α,3α-Dihydroxy-5α-stigmast-22-en-6-one 2,3-acetonide 8**

The 2α,3α-diol (6.0 g, 0.014 mol) was dissolved in a mixture of $CH_2Cl_2$ (60 $cm^3$), $CH_3CN$ (60 $cm^3$) and 2,2-dimethoxypropane (20 $cm^3$) and CSA (0.1 g) was added. The mixture was stirred for 1 h at room temperature. After complete reaction had been observed as indicated by TLC, solid $NaHCO_3$ (1.0 g) was added. The mixture was evaporated, and the residue was dissolved in $CH_2Cl_2$ (3 × 100 $cm^3$) and transferred to a separatory funnel. The solution was washed successively with water (100 $cm^3$), saturated aq. $NaHCO_3$ (2 × 100 $cm^3$), brine (100 $cm^3$), dried ($MgSO_4$), filtered and evaporated. The yield of acetonide after drying *in vacuo* was 6.42 g (98%). A small sample was recrystallized from acetone; mp 164–166 °C [lit.,[11c] 158–159 °C (from 99% EtOH)]; TLC: $CH_2Cl_2$–EtOAc (7:3) $R_F$ = 0.70; $\delta_H$ 0.67–0.68 (6 H, 18- and 19-$H_3$), 0.78–0.85 (9 H, m, 26-, 27- and 29-$H_3$), 1.01 (3 H, d, *J* 6.6, 21-$H_3$), 1.33 (3 H, s, acetonide Me), 1.49 (3 H, s, acetonide Me), 2.3 (1 H, dd, *J* 1.29 and 4.2, 7β-H), 2.5 (1 H, dd, *J* 12.6 and 4.2, 7α-H), 4.09 (1 H, m, 2-H), 4.26 (1 H, d, *J* 2.7, 3-H), 5.05 (1 H, dd, *J* 15.0 and 8.4, 22-H) and 5.11 (1 H, dd, *J* 15.0 and 8.4, 23-H); $\delta_C$ 211.5, 137.9, 129.6, 107.8. 72.3, 72.1, 56.8, 55.8, 53.4, 51.5, 51.2, 46.9, 42.7, 42.5, 41.1, 40.4, 39.2, 37.5, 31.8, 28.7, 28.6, 26.5, 25.4, 24.0, 22.6, 21.1(2), 21.0, 18.9, 12.6, 12.2 and 12.1; $\nu_{max}$(KBr)/$cm^{-1}$ 2957, 2869 and 1709.

**(22*S*,23*S*,24*S*)-2α,3α,22,23-Tetrahydroxy-5α-stigmastan-6-one 2,3-acetonide 9**

A mixture consisting of the above acetonide (5.53 g, 0.0114 mol), NMO (6.68 g, 0.057 mol, 5 mol equiv.), THF (100 $cm^3$), water (25 $cm^3$), $NaHCO_3$ (0.479 g, 0.0057 mol, 0.5 mol equiv.), $MeSO_2NH_2$ (1.09 g, 0.0114 mol, 1 mol equiv.) and $OsO_4$ (7.2 $cm^3$, 2.5% in Bu$^t$OH, 0.05 mol equiv.) was stirred for 6 days at 40 °C, after which TLC (hexane–EtOAc, 7:3) showed almost complete disappearance of the starting material ($R_F$ = 0.56), and appearance of (22*S*,23*S*)-homocastasterone 2,3-acetonide **9** ($R_F$ = 0.27) and (22*R*,23*R*)-homocastasterone 2,3-acetonide ($R_F$ = 0.14). Solid $K_2CO_3$ (1.58 g, 0.0114 mol) was added,

followed by aq. $NaHSO_3$ [4.75 g, 0.0456 mol, 4 mol equiv. in water (25 $cm^3$)], and the mixture was stirred for another 5 h. The solvent was evaporated off and the residue was extracted with EtOAc (3 × 200 $cm^3$), washed successively with 5% aq. $NaHSO_3$ (200 $cm^3$), saturated aq. $NaHCO_3$ (200 $cm^3$) and brine (200 $cm^3$), dried ($MgSO_4$) and filtered. The filtrate was evaporated, and dried *in vacuo*. The crude diol **9** (yield 5.9 g, 98%) was used as such for the next step.

**2α,3α-Dihydroxy-6-oxo-23,24-dinorcholan-22-al 2,3-acetonide 10**

In a flask protected from moisture, crude diol **9** (4.50 g, ~95% diol, 8.67 mmol) was dissolved in freshly distilled THF (200 $cm^3$). To this solution were added pyridine (1 $cm^3$), solid $NaHCO_3$ (0.728 g, 8.67 mmol), and finally $H_5IO_6$ (1.98 g, 8.67 mmol). The mixture was stirred at room temperature under argon. The mixture became turbid almost immediately. After 24 h TLC (hexane–EtOAc, 7:3) showed the complete disappearance of diol **9**. The mixture was filtered in order to remove the precipitated $HIO_3$. The solid was rinsed with THF and the combined washings and filtrate were concentrated to leave an oil. Freshly prepared 5% aq. $NaHSO_3$ (100 $cm^3$) was added and the mixture was stirred for 30 min. Then 10% aq. $Na_2CO_3$ was added and the mixture was stirred for another 30 min.

The product was extracted with $CH_2Cl_2$ (3 × 100 $cm^3$) and the extract was washed successively with 5% saturated aq. $NaHCO_3$ (100 $cm^3$) and brine (100 $cm^3$) and dried ($MgSO_4$). Filtration, and evaporation of filtrate under reduced pressure yielded product (3.50 g). The aldehyde was purified by chromatography using hexane–EtOAc gradient (95:5 to 70:30). The yield of pure aldehyde **10** amounted to 2.86 g (82%). A sample was recrystallized from $CH_2Cl_2$; mp 198–200 °C; $\delta_H$ 0.67 (3 H, s, 18-$H_3$), 0.69 (3 H, s, 19-$H_3$), 1.12 (3 H, d, *J* 6.6, 21-$H_3$), 1.32 (3 H, s, acetonide Me), 1.49 (3 H, s, acetonide Me), 2.08–2.14 (1 H, m, 7α-H), 2.29–2.34 (1 H, m, 7β-H), 2.36 (1 H, m, 20-H), 2.53 (1 H, dd, *J* 12.3 and 3.9, 5α-H), 4.09 (1 H, m, 2-H), 4.26 (1 H, br s, 3-H) and 9.56 (1 H, d, *J* 3.3, 22-H); $\delta_C$ 211.0, 204.6, 107.9, 72.2, 70.1, 55.9, 53.3, 53.2, 51.4, 50.9, 49.3, 46.8, 41.1, 41.0, 38.9, 37.4, 28.6, 26.9, 26.5, 24.3, 22.5, 21.0, 13.4, 12.7 and 12.3; $\nu_{max}$(KBr)/$cm^{-1}$ 2978, 2875, 1700, 1697, 1449, 1366, 1239, 1215 and 1055; HRMS (CI) [Found: $(M + H)^+$, 403.2865. Calc. for $C_{25}H_{39}O_4$: *m/z*, 403.2838].

**2α,3α-Dihydroxy-6-oxo-23,24-dinorcholan-22-al 2,3-acetonide 10 *via* ozonolysis**

2α,3α-Dihydroxystigmast-22-en-6-one 2,3-acetonide **8** (500 mg, 1.03 mmol) was dissolved in a mixture of $CH_2Cl_2$ (47 $cm^3$) and pyridine (3 $cm^3$). The solution was cooled to −78 °C, flushed with a stream of nitrogen, and then subjected to ozonolysis (Welsbach ozone generator) until a deep blue colour formed (10 min). The system was flushed with oxygen, and then activated (acid-washed) zinc dust (800 mg) was added, followed by water (3 $cm^3$). The reaction mixture was allowed to warm up to room temperature overnight. TLC indicated the absence of starting alkene and the presence of aldehyde **10** and the corresponding carboxylic acid in approximately 1:1 ratio. The mixture was washed successively with water (50 $cm^3$) and brine (50 $cm^3$), dried ($MgSO_4$), filtered and evaporated. The residue was purified by chromatography. The first product to be eluted was the aldehyde **10** (200 mg, 48%). The second major product was 2α,3α-dihydroxy-6-oxo-23,24-dinorcholan-22-oic acid 2,3-acetonide (130 mg, 30%); mp 237–239 °C (from hexane–$CH_2Cl_2$); $\delta_H$ 0.625 (6 H, s, 18-and 19-$H_3$), 1.18 (3 H, d, *J* 6.9, 21-$H_3$), 1.29 (3 H, s, acetonide Me), 1.45 (3 H, s, acetonide Me), 1.92–2.00 (3 H, m), 2.05–2.12 (1 H, m, 7α-H), 2.26 (1 H, dd, *J* 12.9 and 3.9, 7β-H), 2.34–2.37 (1 H, m, 20-H), 2.50 (1 H, dd, *J* 12.3 and 3.6, 5α-H), 4.05 (1 H, m, 2-H) and 4.23 (1 H, s, 3-H); $\delta_C$ 211.2, 182.3, 107.9, 72.2, 72.1, 56.2, 53.2, 51.4, 46.7, 42.9, 42.4(2), 41.0, 39.1, 37.4, 28.5, 27.0, 26.4, 23.9, 22.5, 21.0, 16.9, 12.6 and 12.1.

**3-Isopropylbut-2-enolide**

A solution of 3-methylbutan-2-one (43 g, 0.50 mol) in methanol (500 $cm^3$) was cooled to −30 °C. Bromine (80.0 g, 0.50 mol) was added dropwise over a period of 30 min. The reaction mixture was allowed to warm to ~25 °C. At this temperature, the solution became colourless and was immediately added to ice-cold aq. 5% $NaHCO_3$ (2 $dm^3$). The mixture was extracted with hexanes (3 × 200 $cm^3$). The combined extracts were dried over anhydrous $MgSO_4$, filtered and concentrated to a volume of 75 $cm^3$. This crude lachrymatory product was then added to a refluxing solution of anhydrous KOAc (80.0 g, 0.82 mol) in acetone (2 $dm^3$). After 18 h the mixture was poured into water (1 $dm^3$) and then the phases were separated. The aqueous phase was further extracted with diethyl ether (3 × 400 $cm^3$). The combined organic phases were washed with 5% aq. $NaHCO_3$, dried ($MgSO_4$) and filtered. Most of the ether was removed and the residual liquid was distilled (bp 62–65 °C at 0.2 mmHg) to give 1-acetoxy-3-methylbutan-2-one (30.0 g, 42%); $\delta_H$ 1.15 (6 H, d, *J* 7, CH$Me_2$), 2.21 (3 H, s, OCOMe), 2.71 (1 H, septet, *J* 7, methine H) and 4.75 (2 H, s, methylene H).

Triethylphosphonoacetate(ethyl diethoxyphosphoryacetate) (46.0 g, 0.205 mol) was added over a period of 30 min to a cooled suspension of 60% NaH (8.2 g, 0.205 mol) in THF (200 $cm^3$), while the temperature was maintained at below 30 °C. The resulting solution was cooled to 15 °C and the ketoacetate (29.5 g, 0.205 mol) was added over a period of 5 min. After 30 min at 25 °C, the solution was poured into 10% aq. $Na_2SO_4$ (500 $cm^3$) and the mixture was extracted with diethyl ether (3 × 100 $cm^3$). The combined extracts were washed (10% $Na_2SO_4$), dried ($MgSO_4$), and concentrated under reduced presure. The yield of the crude product, consisting of the diester and lactone in the ratio 9:1, was 47 g. To this product was added 6 mol $dm^{-3}$ aq. KOH (250 $cm^3$). After storage for 24 h at 25 °C, the reaction mixture became homogeneous and was chilled and acidified to pH 1 with 6 mol $dm^{-3}$ HCl. After being stirred for 1 h the solution was extracted with ethyl acetate (3 × 200 $cm^{-3}$). The organic layer was washed with 10% aq. $Na_2SO_4$ (2 × 200 $cm^3$), dried ($MgSO_4$), and concentrated under reduced pressure, to yield a mixture (21 g) consisting of the butenolide and other more polar products. The 3-isopropylbutenolide (7.2 g) was obtained pure by chromatography (28% from the keto acetate); $\delta_H$ 1.20 (6 H, d, *J* 7, CH$Me_2$), 2.70 (1 H, m, methine H), 4.76 (2 H, d, *J* 1.2, methylene H) and 5.79 (1 H, s, vinyl H); $\delta_C$ 175.6, 113.3, 104.9, 71.4, 27.9 and 20.4; $\nu_{max}$(KBr)/$cm^{-1}$ 2959, 3936, 2878, 1784, 1748 and 1632.

**(22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-6-oxo-5α-sitost-24(28)-en-29-oic acid γ-lactone 2,3-acetonide 11**

A 0.50 mol $dm^{-3}$ solution of LDA in THF was prepared by dissolution of diisopropylamine (2.78 g, 27.5 mmol) in anhydrous THF (34 $cm^3$) at −40 °C, and addition of n-butyllithium (1.6 mol $dm^{-3}$; 17.2 $cm^3$) all under argon. After 5 min, a solution of 3-isopropylbutenolide (3.48 g, 27.6 mmol, 6 mol equiv.) in anhydrous THF (40 $cm^3$) was added, *via* a cannula, during 5 min. A clear yellow solution resulted immediately. The mixture was stirred for 30 min at −40 to −50 °C, then it was cooled to −78 °C and a solution of 2α,3α-dihydroxy-6-oxo-23,24-dinorcholan-22-al 2,3-acetonide **10** (1.85 g, 4.59 mmol) in anhydrous THF (40 $cm^3$) was added *via* a cannula, during 5 min. The reaction mixture was stirred for 14 h at below −75 °C, after which TLC ($CH_2Cl_2$–EtOAc, 8:2) indicated complete disappearance of substrate **10**. With the reaction mixture's temperature still at −78 °C, saturated aq. $NH_4Cl$ (25 $cm^3$) was added in one portion. The mixture was allowed to come to room temperature overnight. THF was removed under reduced pressure and the residue was extracted with $CH_2Cl_2$ (3 × 100 $cm^3$) and the extract was washed successively with water (100 $cm^3$), saturated aq. $NaHCO_3$ (100 $cm^3$) and brine (100 $cm^3$), dried ($MgSO_4$), and filtered. The solvent was evaporated off and the residue was dried

*in vacuo*. The crude product **11** (~5.3 g) was purified by chromatography ($CH_2Cl_2$–EtOAc). Isopropylbutenolide (2.75 g) was obtained first and further elution gave a mixture consisting of the minor 22*R*,23*S* condensation isomer and possibly the product corresponding to the condensation between the aldehyde and other resonance form of the anion of 3-isopropylbutenolide. The yield of this mixture was 0.18 g (9.3%).

The last product eluted was the desired (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-6-oxo-5α-sitost-24(28)-en-29-oic acid γ-lactone 2,3-acetonide **11** (1.94 g, 80%); mp 240–242 °C (from hexane–$CH_2Cl_2$); $\delta_H$ 0.66 (3 H, s, 18-$H_3$), 0.68 (3 H, s, 19-$H_3$), 1.11 (3 H, d, *J* 6.6, 21-$H_3$), 1.14 (3 H, d, *J* 6.9, 26-$H_3$), 1.24 (3 H, d, *J* 6.3, 27-$H_3$), 1.32 (3 H, s, acetonide Me), 1.48 (3 H, s, acetonide Me), 2.30 (1 H, dd, *J* 12.9 and 4.2, 7β-H), 2.55 (2 H, m, 5α-and 25-H), 3.88 (1 H, d, *J* 10.5, 22-H), 4.08 (1 H, m, 2-H), 4.26 (1 H, br s, 3-H), 4.78 (1 H, s, 23-H) and 5.79 (1 H, s, 28-H); $\delta_C$ 211.3, 177.5, 173.2, 114.7, 107.8, 85.8, 72.2, 72.1, 71.2, 56.3, 53.1, 52.6, 51.4, 46.8, 42.8, 42.4, 41.9, 41.0, 39.2, 37.5, 28.6, 28.1, 27.8, 26.5, 23.9, 22.5, 21.7, 21.0, 20.3, 12.9, 12.6 and 11.7; $\nu_{max}$(KBr)/cm$^{-1}$ 3592, 3470, 2966, 2942, 2869, 1752, 1694 and 1631.

A sample of compound **11** was deprotected by dissolution (50 mg, 0.094 mmol) in TFA (1 cm$^3$), methanol (5 cm$^3$) and water (1 cm$^3$). After 1 h the reaction mixture was evaporated under reduced pressure. Crystals of (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-6-oxo-5α-sitost-24(28)-en-29-oic-acid γ-lactone were obtained from methanol; mp 249–253 °C; $\delta_H$ 0.71 (3 H, s, 18-$H_3$), 0.76 (3 H, s, 19-$H_3$), 1.13 (3 H, d, *J* 5.4, 21-$H_3$), 1.16 (3 H, d, *J* 6.6, 26-$H_3$), 1.26 (3 H, d, *J* 6.6, 27-$H_3$), 2.30 (1 H, dd, *J* 13.2 and 4.5, 7β-H), 2.61 (1 H, m, 25-H), 2.70 (1 H, dd, *J* 12.9 and 3.0, 5α-H), 3.76 (1 H, br d, 2-H), 3.90 (1 H, d, *J* 10.5, 22-H), 4.03 (1 H, br s, 3-H), 4.88 (1 H, s, 23-H) and 5.82 (1 H, s, 28-H); $\delta_C$ 211.7, 177.7, 114.7, 105.4, 86.1, 71.2, 68.4, 68.3, 56.4, 53.6, 52.7, 50.7, 46.7, 42.9, 42.5, 42.12, 40.1, 39.4, 37.6, 28.2, 27.8, 26.9, 26.2, 21.7, 21.2, 20.3, 13.5, 13.1 and 11.8; $\nu_{max}$(KBr)/cm$^{-1}$ 3445, 2943, 2871, 1753, 1704, 1632, 1460, 1386, 1260, 1173, 1048, 966 and 876. The assigned structure was confirmed by X-ray crystallography.

**(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-6-oxo-5α-sitostan-29-oic acid γ-lactone 2,3-acetonide 12**

Compound **11** (1.42 g, 2.68 mmol) was hydrogenated in a Parr hydrogenator at 50 p.s.i. and ambient temperature, by first placing 10% palladium on activated carbon (0.71 g) in the flask, followed by compound **11** previously dissolved in anhydrous THF (150 cm$^3$), and pyridine (0.25 cm$^3$). The mixture was allowed to react for 14 h. After disappearance of substrate **11** as indicated by TLC the catalyst was removed by gravity filtration and rinsed with THF. Removal of the solvent from the filtrate gave solid (1.50 g), which was purified by chromatography ($CH_2Cl_2$–EtOAc; gradient elution).

The first product eluted was the minor (24*R*) isomer of compound **12** (0.23 g, 17%); $\delta_H$ 0.68 (3 H, s, 18-$H_3$), 0.69 (3 H, s, 19-$H_3$), 0.92 (3 H, d, *J* 7.5, 26-$H_3$), 0.95 (3 H, d, *J* 6.9, 27-$H_3$), 1.04 (3 H, d, *J* 6.9, 21-$H_3$), 1.34 (3 H, s, acetonide Me), 1.51 (3 H, s, acetonide Me), 2.21–2.35 (2 H, m, 7-$H_2$), 2.42–2.47 (1 H, m, 28-H), 2.52–2.57 (1 H, dd, *J* 12.3 and 8.1, 28-H), 3.60 (1 H, dd, *J* 8.4 and 4.2, 22-H), 4.10 (1 H, m, 2-H), 4.28 (1 H, br s, 3-H) and 4.36 (1 H, d, *J* 5.4, 23-H); $\delta_C$ 211.2, 177.4, 107.9, 81.4, 74.0, 72.2, 72.1, 56.1, 53.2, 53.1, 51.4, 46.7, 43.9, 43.4, 42.4, 41.5, 41.0, 39.2, 37.4, 31.0, 29.9, 28.5, 27.6, 26.5, 24.1, 22.5, 21.0, 20.3, 18.4, 13.9, 12.6 and 11.8; $\nu_{max}$(KBr)/cm$^{-1}$ 3486, 2947, 2872, 1771 and 1709.

The second compound eluted was the major product **12** (1.18 g, 83%); mp 230–232 °C (plates from $CH_2Cl_2$); $\delta_H$ 0.67 (6 H, s, 18- and 19-$H_3$), 0.92–0.95 (6 H, 2 × d, *J* 6.9 and 6.6, 26- and 27-$H_3$), 1.01 (3 H, d, *J* 6.3, 21-$H_3$), 1.34 (3 H, s, acetonide Me), 1.51 (3 H, s, acetonide Me), 1.57–1.78 (3 H, m), 1.93–2.06 (3 H, m), 2.09–2.14 (1 H, m), 2.19–2.34 (2 H, m), 2.52–2.65 (2 H, m, 5α- and 28-H), 3.57–3.61 (1 H, m, 22-H), 4.11 (1 H, m, 2-H) and 4.22–4.28 (2 H, m, 3- and 23-H); $\delta_C$ 211.3, 176.7, 107.8, 85.6, 74.5, 72.2, 72.1, 56.4, 53.1, 52.2, 51.4, 46.8, 43.2, 42.6, 42.4, 41.0, 39.2, 39.1, 37.5, 31.0, 30.5, 28.6, 27.9, 26.5, 23.8, 22.5, 21.0, 20.1, 18.6, 12.6, 12.5 and 11.7; $\nu_{max}$(KBr)/cm$^{-1}$ 3588, 3460, 2942, 2869 and 1774.

**(22*R*,23*R*)-2α,3α,6β,22,23,29-Hexahydroxy-5α-sitostane 2,3-acetonide 13**

A solution of keto lactone **12** (1.72 g, 3.24 mmol) in anhydrous THF (50 cm$^3$) was added *via* a cannula to a suspension of $LiAlH_4$ (0.738 g, 6 mol equiv.) in THF (60 cm$^3$) at ambient temperature, all under argon. The reaction mixture was stirred for 2 h, and then heated to reflux overnight. TLC indicated the disappearance of keto lactone **12**, and the formation of hexaol derivative **13** (6β-OH) and its isomer (6α-OH) in an approximately 6:1 ratio. The reaction mixture was chilled and then 15% aq. NaOH (20 cm$^3$), followed by water (10 cm$^3$), was added in order to destroy excess of hydride. The mixture was extracted with diethyl ether (3 × 100 cm$^3$). The organic phase was washed successively with water (50 cm$^3$), saturated aq. $NaHCO_3$ (100 cm$^3$) and brine (100 cm$^3$), dried ($MgSO_4$), filtered and evaporated. After being dried *in vacuo*, compound **13** was obtained (1.70 g, 99% for the two isomers).

**(22*R*,23*R*)-2α,3α,6β,22,23,29-Hexahydroxy-5α-sitostane 2,3;22,23-diacetonide 14**

To the above tetraol **13** (mixture of isomers 1.67 g, 3.11 mmol) dissolved in anhydrous acetone (100 cm$^3$) was added CSA (0.072 g). The mixture was stirred at room temperature under nitrogen for 24 h. To this mixture was added solid $NaHCO_3$ (2.0 g). The acetone was evaporated off, and the residue was extracted with EtOAc (300 cm$^3$). The organic solution was washed successively with water (50 cm$^3$), saturated aq. $NaHCO_3$ (100 cm$^3$) and brine (100 cm$^3$), dried ($MgSO_4$), filtered and the filtrate was evaporated to give compound **14** (1.7 g, 96%); $\delta_H$ 0.64 (3 H, s, 18-$H_3$), 0.73 (3 H, s, 19-$H_3$), 0.82 (3 H, d, *J* 6.9, 26-$H_3$), 0.93 (3 H, d, *J* 6.6, 27-$H_3$), 0.97 (3 H, d, *J* 6.0, 21-$H_3$), 1.34 (3 H, s, acetonide Me), 1.36 (6 H, br s, acetonide Me), 1.49 (3 H, s, acetonide Me), 2.47–2.56 (1 H, dd, *J* 15.3 and 2.7, 5α-H), 3.31 (1 H, m, 6-H), 3.50–3.87 (4 H, m, 22- and 23-H and 29-$H_2$), 4.10 (1 H, m, 2-H) and 4.26 (1 H m, 3-H); $\delta_C$ 107.6, 107.5, 81.3, 78.5, 72.9, 72.5, 70.4, 62.5, 55.8, 53.4, 53.2, 46.5, 45.1, 42.4, 41.7(2), 39.5, 36.2, 33.9, 30.0, 29.7, 28.6, 28.4, 27.8, 27.2, 27.1, 26.5, 25.8, 24.0, 20.8, 20.7, 18.0, 12.5 and 11.7.

**(22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-6-oxo-5α-sitostan-29-al 2,3;22,23-diacetonide 15**

$CrO_3$ (1.48 g, 14.8 mmol) was added in portions to a flask containing dry pyridine (15 cm$^3$) under nitrogen. The yellow $CrO_3$–pyridine complex was allowed to form for 30 min. The complex was diluted with dry $CH_2Cl_2$ (100 cm$^3$), then a solution of diol **14** (1.70 g, 2.95 mmol) in $CH_2Cl_2$ (50 cm$^3$) was added *via* a cannula. The mixture soon became dark brown. The mixture was stirred for 48 h at room temperature, after which TLC indicated the disappearance of substrate **14** and the complete formation of aldehyde **15**. The mixture was filtered through a bed of Florisil, the filtrate was evaporated, and the residue was purified by chromatography (hexane–EtOAc, 100:0 to 50:50 gradient) to afford compound **15** (1.53 g, 91%); mp 156–158 °C (from hexane); $\delta_H$ 0.65 (3 H, s, 18-$H_3$), 0.66 (3 H, s, 19-$H_3$), 0.84 (3 H, d, *J* 6.9, 26-$H_3$), 0.93 (3 H, d, *J* 6.9, 27-$H_3$), 0.97 (3 H, d, *J* 6.0, 21-$H_3$), 1.31 (3 H, s, acetonide Me), 1.33 (6 H, br s, acetonide Me), 1.50 (3 H, s, acetonide Me), 1.89–2.07 (5 H, m), 2.07–2.15 (1 H, m, 7α-H), 2.27–2.39 (2 H, m, 7β and 28-H), 2.50–2.63 (1 H, m, 5α-H), 3.70 (1 H, d, *J* 8.4, 22-H), 3.79 (1 H, dd, *J* 8.1 and 5.1, 23-H), 4.08 (1 H, m, 2-H), 4.26 (1 H, br s, 3-H) and 9.78–9.81 (1 H, m, 29-H); $\delta_C$ 211.4, 202.5, 107.9(2), 95.1, 81.0, 77.9, 72.3, 72.1, 56.3, 53.3, 53.2, 51.4, 46.8, 42.8, 41.2, 41.1, 39.2, 37.6, 35.9, 35.6, 29.4, 28.6, 27.5, 27.1, 26.9, 26.5, 23.8, 22.5, 21.1, 20.8, 18.4, 12.6, 12.5 and 11.7; $\nu_{max}$(KBr)/cm$^{-1}$ 2944, 2872 and 1710.

**(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-5α-campestan-6-one 2,3;22,23-diacetonide 16**

The aldehyde **15** (0.834 g, 1.45 mmol) as a solution in dry degassed benzene (83 $cm^3$) was heated to reflux, in the presence of tris(triphenylphosphine)rhodium(I) chloride (2.02 g, 1.5 mol equiv.) under argon for 5 h. During the reaction, a red-orange colour developed. After cooling, the mixture was initially filtered through a bed of Florisil, and this was followed by a rinse with hexane–EtOAc (6:4; 200 $cm^3$). The solvent was evaporated off from the filtrate and the residue was purified by chromatography (hexane–EtOAc; 100:0 to 80:20 gradient) to yield compound **16** (0.570 g, 72%); mp 149–151 °C (from hexane); $\delta_H$ 0.65 (3 H, s, 18-$H_3$), 0.67 (3 H, s, 19-$H_3$), 0.85 (3 H, d, *J* 6.9, 26-$H_3$), 0.87 (3 H, d, *J* 6.6, 27-$H_3$), 0.93 (3 H, d, *J* 6.6, 28-$H_3$), 0.98 (3 H, d, *J* 6.6, 21-$H_3$), 1.34, 1.37, 1.51 and 1.56 (12 H, 4 × s, acetonide methyls), 1.93–2.04 (5 H, m), 2.09–2.15 (1 H, m, 7α-H), 2.31 (1 H, dd, *J* 12.9 and 4.2, 7β-H), 2.55 (1 H, dd, *J* 12.6 and 3.9, 5α-H), 3.73 (1 H, dd, *J* 8.4 and 4.2, 23-H), 3.82 (1 H, d, *J* 8.4, 22-H), 4.09 (1 H, m, 2-H) and 4.27 (1 H, br s, 3-H); $\delta_C$ 211.5, 107.9, 107.6, 80.4, 79.1, 72.3, 72.1, 56.4, 53.3, 53.2, 51.5, 46.9, 42.8, 42.5, 41.1, 40.6, 39.1, 37.6, 36.1, 30.6, 29.7, 28.6, 27.8, 27.3, 26.5, 23.9, 22.5, 21.1, 18.4, 12.7, 11.7 and 9.9; $\nu_{max}$(KBr)/$cm^{-1}$ 2939, 2874 and 1705.

**(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-5α-campestan-6-one (castasterone) 17**

Compound **16** was dissolved in methanol (10 $cm^3$)–water (1 $cm^3$), and TFA (50 mg, 0.092 mmol) was added. After 1 h the mixture was evaporated and the residue was dried *in vacuo* to yield compound **17** (42 mg, 98%). Crystals were obtained from EtOAc; mp 251–253 °C [lit.,[18] 258–260 °C (from $CHCl_3$–MeOH)]; $\delta_H$ 0.68 (3 H, s, 18-$H_3$), 0.76 (3 H, s, 19-$H_3$), 0.84 (3 H, d, *J* 6.6, 26-$H_3$), 0.91 (3 H, d, *J* 6.6, 27-$H_3$), 0.94 (3 H, d, *J* 6.3, 28-$H_3$), 0.97 (3 H, d, *J* 6.6, 21-$H_3$), 2.30 (1 H, dd, *J* 12.9 and 4.2, 7β-H), 2.67 (1 H, dd, *J* 15.6 and 3.0, 5α-H), 3.55 (1 H, d, *J* 8.1, 22-H), 3.72 (1 H, d, *J* 9, 23-H), 3.78 (1 H, m, 2-H) and 4.05 (1 H, br s, 3-H); $\delta_C$ [$(CD_3)_2SO$] 211.1, 79.1, 78.3, 72.9, 71.7, 67.4, 67.1, 55.9, 51.7, 50.2, 45.9, 42.1, 41.7, 39.9, 39.1, 37.0, 36.6, 30.1, 27.0, 26.6, 23.3, 20.7, 20.5, 13.2, 11.8, 11.6 and 10.0; $\nu_{max}$(KBr)/$cm^{-1}$ 3400, 2944, 2870, 1708, 1450, 1382, 1260, 1084, 1042, 992 and 978.

**(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-7-oxa-7a-homo-5α-campestan-6-one (brassinolide) 1**

Peroxytrifluoroacetic acid was generated *in situ* by slow addition of 30% aq. $H_2O_2$ (0.63 $cm^3$, 5.56 mmol) to trifluoroacetic anhydride (TFAA) (4 $cm^3$, 28.3 mol) at 0 °C, and the stirred mixture was allowed to react for 30 min. Simultaneously, castasterone diacetonide **16** (400 mg, 0.681 mmol) was dissolved in chloroform (30 $cm^3$) and the solution was treated with TFA (5 $cm^3$) for 30 min at room temperature. The latter solution was added dropwise to the peroxytrifluoroacetic acid solution *via* a cannula. The mixture was allowed to react for 2 h at room temperature. TLC ($CHCl_3$–$Pr^iOH$, 9:1) indicated the disappearance of protected castasterone **16** ($R_F$ = 0.78) and castasterone **17** ($R_F$ = 0.20), and the appearance of brassinolide **1** ($R_F$ = 0.13) along with some trifluoroacetic esters ($R_F$ = 0.33 and 0.41). The mixture was diluted with chloroform (50 $cm^3$), and washed successively with water (25 $cm^3$) and 10% aq. $NaHSO_3$ (2 × 25 $cm^3$), and the chloroform was evaporated off. To the residue were added methanol and solid $Na_2CO_3$ (50 mg) and the mixture was stirred overnight. The methanol was evaporated off and the residue was dissolved in chloroform (200 $cm^3$); the solution was washed successively with water (25 $cm^3$), 1 mol $dm^{-3}$ HCl (2 × 10 $cm^3$), saturated aq. $NaHCO_3$ (25 $cm^3$) and brine (25 $cm^3$), dried ($MgSO_4$) and filtered, and the filtrate was evaporated. The crude material (320 mg, 97%) was purified by chromatography ($CHCl_3$–$Pr^iOH$, 90:10 to 80:20 gradient). Brassinolide **1** (300 mg, 92%) was isolated as a mixture with its 6-oxa-7a-homo-isomer (9:1), as indicated by NMR spectroscopy. Pure brassinolide **1** was obtained by slow crystallization from methanol (200 mg, 62%); mp 268–270 °C (lit.,[1] 273–275 °C); $\delta_H$ 0.72 (3 H, s, 18-$H_3$), 0.84 (3 H, d, *J* 6.9, 26-$H_3$), 0.90 (3 H, d, *J* 6.6, 27-$H_3$), 0.92 (3 H, s, 19-$H_3$), 0.95 (3 H, d, *J* 6.0, 28-$H_3$), 0.97 (3 H, d, *J* 6.3, 21-$H_3$), 1.20–2.20 (several H, m, not assigned), 3.11 (1 H, dd, *J* 12.0 and 4.5, 5α-H), 3.50 (1 H, dd, *J* 7.8 and 3.3, 23-H), 3.69–3.73 (2 H, m, 2-and 7a-H), 4.05 (1 H, d, *J* 13.2, 7a-H), 4.1 (1 H, br s, 3-H); $\delta_C$([$^2H_5$]pyridine) 176.6, 74.2, 73.1, 70.3, 68.8, 68.4, 60.3, 58.4, 52.9, 51.6, 42.8, 41.7, 41.2, 40.1, 39.7, 38.6, 38.1, 33.2, 31.4, 28.1, 24.9, 22.6, 21.3, 21.0, 15.9, 12.7, 11.9 and 10.9; $\nu_{max}$(KBr)/$cm^{-1}$ 3450, 2965, 2941, 2868, 1699, 1464, 1382, 1331, 1185, 1064, 1025 and 982; HRMS (CI) [Found: $(M + 1)^+$, 481.3498. Calc. for $C_{28}H_{49}O_6$: *m/z*, 481.3516]. The assigned structure was confirmed by X-ray crystallography.

## Acknowledgements

This investigation was supported by a grant from the NIH (GM 47165) and an Industrial Research Fellowship from the National Sciences and Engineering Research Council of Canada through Beak Consultants Limited, Ontario, Canada.

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*Paper* 5/03686K
*Received* 8*th June* 1995
*Accepted* 8*th August* 1995