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SYNTHESIS OF (22*S*, 23*S*)-HOMOBRASSINOLIDE AND BRASSINOLIDE FROM STIGMASTEROL†

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Abstract—(22*S*, 23*S*)-Homobrassinolide (2α, 3α, 22*S*, 23*S*-tetrahydroxy-24*S*-ethyl-B-homo-7-oxa-5α-cholestan-6-one) and brassinolide (2α, 3α, 22*R*, 23*R*-tetrahydroxy-24*S*-methyl-B-homo-7-oxa-5α-cholestan-6-one) were synthesized from stigmasterol and shown to promote plant growth.

In 1979 Grove *et al.* isolated 4 mg of a new steroid named brassinolide from 40 kg of bee-collected pollen of the rape plant, *Brassica napus* L. as a plant growth promoter.[1] Its structure was established as 2α, 3α, 22*R*, 23*R*-tetrahydroxy-24*S*-methyl-B-homo-7-oxa-5α-cholestan-6-one **1a** by X-ray analysis.[1] The structure **1a** as B-seco steroidal lactone with four OH groups is unique enough to make it an attractive target of organic synthesis. An additional interest lies in its remarkable biological activities in promoting division and elongation of plant cells.[2] We therefore began our works on brassinolide and its analogs in order to develop simple synthetic routes to them. In view of the scarcity of the natural material, synthetic works are absolutely necessary in evaluating the effect of these steroids on plant growth.

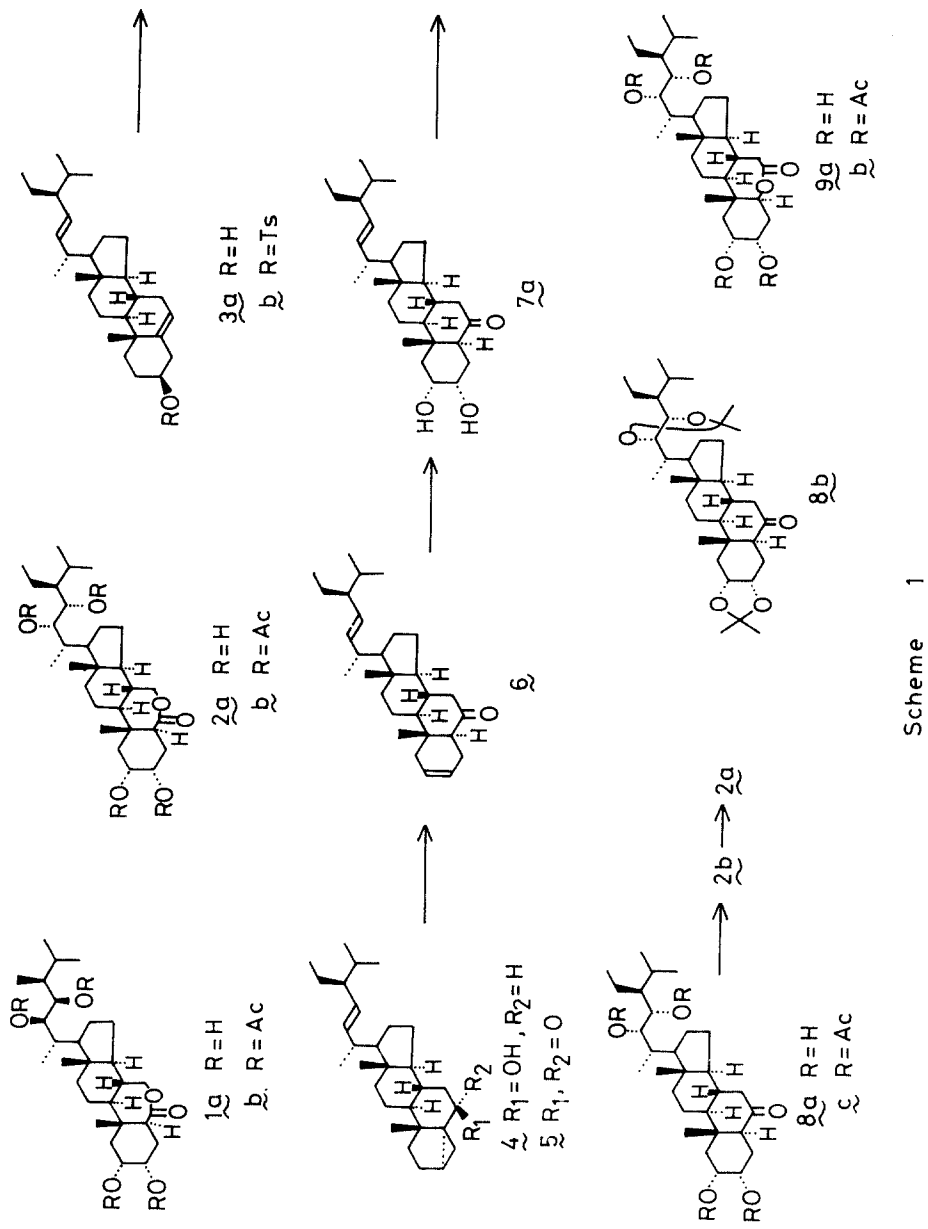
Synthesis of (22*S*, 23*S*)-*homobrassinolide.* The first phase of our work was to develop a simple and efficient synthesis of a brassinolide analog with all of the functional groups of brassinolide itself. We envisaged (22*S*, 23*S*)-homobrassinolide **2a** as our target molecule employing stigmasterol **3a** as the starting material. The reason for this decision was the ready availability of **3a** as well as the common (24*S*)-configuration of **3a** and **1a**. The conversion of **3a** to **2a** was accomplished as follows (Scheme 1).[3]

Stigmasteryl tosylate **3b** was solvolyzed to give i-stigmasterol **4**.[4] The crude **4** was oxidized with the Jones CrO_3 to give a crystalline ketone **5**. This was heated with p-TsOH in sulfolane[5] to give an unsaturated ketone **6** in 40% yield from stigmasterol **3a**.

Introduction of the four OH groups was effected by OsO_4 oxidation of **6**. The double bond in ring A was oxidized fairly rapidly (5–7 hr) with a catalytic amount of OsO_4 and excess N-methylmorpholine-N-oxide (NMO) in aq acetone[6] to give a highly crystalline diol **7a**. The OsO_4 oxidation of Δ^2-steroid is known to yield 2α, 3α-diol.[7] Further oxidation of **7a** with 1 eq of OsO_4 in C_5H_5N proceeded slowly to give a tetraol **8a** after 1 day. This was converted to a crystalline *bis*-acetonide **8b** in 60% yield from **6**. The hplc analysis of **8b** revealed it to be pure. This implied that the OsO_4 oxidation of the side-chain double bond at C-22 was rather stereoselective under the present condition. Indeed the hplc analysis of a crude reaction product prior to the isolation of **8b** revealed it to contain 86.6% of **8b**. The tetraol **8a** was also obtainable in a single step from **6** by lengthening the reaction time of the OsO_4 oxidation in aq THF with occasional addition of NMO. After 7 to 8 days, the main component of the dark-colored reaction mixture was the tetraol **8a**, which could be purified by chromatography and recrystallization. In a large-scale occasion, the crude **8a** resulting from the single-step oxidation was converted to the crude *bis*-acetonide **8b**, which was analyzed by hplc. The result indicated that the crude acetonide contained two unidentified impurities at R_t 4.8 min (4.8%) and 20.5 min (15.6%) besides the *bis*-acetonide **8b** (R_t 13.2 min; 77%). The crude **8b** could be purified by preparative hplc. In its ^{13}C-NMR spectrum, the pure *bis*-acetonide **8b** exhibited four signals due to C–O (δ 72.1, 72.3, 77.4 and 79.8 ppm), two signals due to O–C–O (δ 106.7 and 107.8 ppm) and a signal due to C=O (δ 211 ppm).

To clarify the stereochemistry of the side-chain portion of the *bis*-acetonide **8b**, an X-ray crystallographic analysis was carried out. A computer-generated perspective drawing of **8b** is shown in Fig. 1. Table 1 shows the torsional angles around C-20, C-21, C-22 and C-23. On the basis of these data coupled with the known absolute stereochemistry of stigmasterol **3a**, (22*S*, 23*S*)-stereochemistry could be assigned to the *bis*-acetonide **8b**. All molecular parameters are consistent with those of related steroidal molecules. Each molecule is so packed that the steroidal nucleus is almost parallel to the a–b plane. There is no intermolecular contact shorter than the corresponding van der Waals distance. It thus became clear that the OsO_4 oxidation stereoselectively generated (22*S*, 23*S*)-glycol system, which is un-

† *Brassinolide and its analogs*, Part I. This work was presented by K. M. as a part of his lecture at the 9th Conference on Isoprenoids, Prague, Czechoslovakia, on 9 September 1981.


1a R=H
b R=Ac
2a R=H
b R=Ac
3a R=H
b R=Ts
4 R1=OH, R2=H
5 R1, R2=O
6
7a
8a R=H
c R=Ac
8b
9a R=H
b R=Ac
2b → 2a

Scheme 1

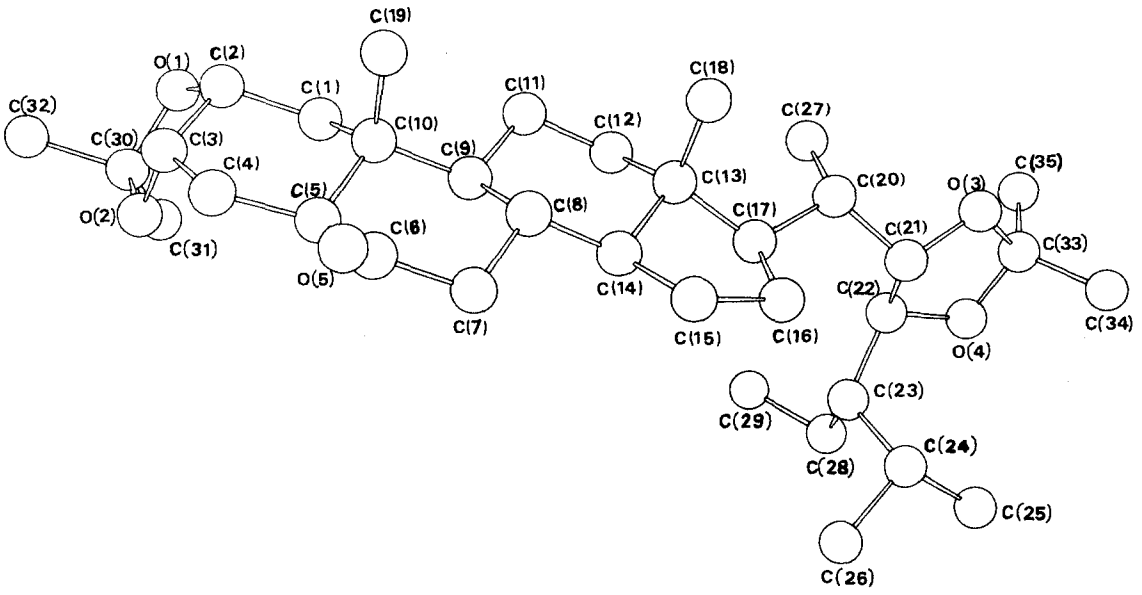


Fig. 1. A computer-generated perspective drawing of the *bis*-acetonide **8b** together with the numbering scheme.

Table 1. Torsional angles around C(20), C(21), C(22) and C(23) atoms (degree). The torsion angle of A(1)–A(2)–A(3)–A(4) is defined as the angle between A(1)–A(2) and A(3)–A(4) (reference) when projected onto the plane normal to A(2)–A(3). Positive and negative signs mean clockwise and counterclockwise direction, respectively.

H(201)-C(20)-C(21)-C(22)	162.3
C(27) -C(20)-C(21)-C(22)	53.2
C(17) -C(20)-C(21)-C(22)	-75.9
H(211)-C(21)-O(3) -C(33)	-98.3
C(20) -C(21)-O(3) -C(33)	143.9
C(22) -C(21)-O(3) -C(33)	19.6
H(221)-C(22)-O(4) -C(33)	-93.3
C(23) -C(22)-O(4) -C(33)	153.9
C(21) -C(22)-O(4) -C(33)	25.9
H(231)-C(23)-C(22)-O(4)	179.5
C(28) -C(23)-C(22)-O(4)	67.4
C(24) -C(23)-C(22)-O(4)	-58.2

fortunately the opposite configuration to that of brassinolide **1a** itself.†

The remaining steps to (22*S*, 23*S*)-homobrassinolide **2a** were straightforward. The tetraol **8a** was converted to a gummy tetraacetate **8c** by treatment with Ac_2O–C_5H_5N in the presence of 4-(N, N-dimethylamino)-pyridine (DMAP). This was submitted to the Baeyer–Villiger oxidation with CF_3CO_3H to give a crystalline tetraacetate **2b** of (22*S*, 23*S*)-homobrassinolide in 66% yield after chromatographic purification. The peracid oxidation of a 6-keto steroid has previously been reported to give this type of ε-lactone as the major product.[8‡] Saponification of **2b** was followed by acidification to give (22*S*, 23*S*)-homobrassinolide **2a**, m.p. 193–194°. The structure **2a** was supported by the spectral data including ^{13}C-NMR. The whole synthetic process was quite reproducible and we could prepare more than 5 g of (22*S*, 23*S*)-homobrassinolide **2a** for biological evaluation. Like brassinolide itself, this analog **2a** showed remarkable activity as a plant growth promoter.[9]

After the completion of our work, a synthesis of the (24*R*)-isomer of brassinolide from ergosterol was reported by Thompson *et al.*[10] Our synthesis has the following three merits compared with their synthesis. (i) The key ketone **6** was obtained in a single step from **5**, while Thompson *et al.* required three steps for the preparation of their ketone corresponding to **6**. (ii) The stoichiometric use of the expensive OsO_4 could be avoided. (iii) Our Baeyer–Villiger oxidation of **8c** with CF_3CO_3H was rapid (1.5 hr for completion), while with *m*-chloroperbenzoic acid (MCPBA) 2 weeks were necessary for completion.

Synthesis of brassinolide. Soon after the publications of Thompson's and our works on brassino steroids,[3,10] two syntheses of brassinolide **1a** itself were reported by Ikekawa *et al.*[11] and by Siddall *et al.*[12] They constructed the dihydroxy side-chain by first generating asymmetry at C-22 and then controlling the stereochemistry at C-23 by OH-directed epoxidation. The clever use of organometallics in their syntheses is indeed noteworthy but may become a draw-back in the practical-scale preparation of **1a**. Our strategy for brassinolide synthesis was to make the chiral side-chain portion first, then to attach it to the steroid nucleus yielding a compound with a double bond at C-22 and finally to oxidize that double bond to give a glycol.[13]

†In our preliminary communication,[3] the tetraol obtained by the OsO_4 oxidation was thought to be a stereoisomeric mixture of **8a** and its (22*R*, 23*R*)-isomer. Reexamination as reported here proved it to be a single isomer. The previously reported[3] homobrassinolide was therefore pure (22*S*, 23*S*)-**2a**.

‡In a small-scale experiment, **2b** was the only isolable product. But in the case of a large-scale preparation, there was also obtained the minor product **9b** of the Baeyer–Villiger oxidation. The properties of **9b** as well as **9a** will be reported later in connection with the synthesis of aza-analogs of brassinolide.

The synthesis of the side-chain portion of the molecule had already been studied in part in connection with our work on the synthesis of faranal, the trail pheromone of the Pharaoh's ant, and the preparation of the optically pure acid **15** had been reported in a preliminary form.[14] Although the (*R*)-acid **15** was previously prepared either by asymmetric synthesis (82% e.e.)[15] or by one-carbon elongation of the lower homolog obtained by resolution,[16] our synthesis starting from optically pure (*R*)-(+)-citronellic acid ensured the high optical purity of our (*R*)-acid **15** (Scheme 2). (*R*)-(+)-Citronellic acid **10** was methylated with LDA and MeI to give **11**. This was reduced with LAH to **12a**. The corresponding tosylate **12b** was again reduced with LAH to an olefin **13**. Treatment of **13** with Ph_2Se_2–H_2O_2–$MgSO_4$ and *t*-BuOOH[17] yielded an allylic alcohol **14**.† Ozonolysis of **14** was followed by oxidative work-up with CrO_3 to give (*R*)-**15**, $[\alpha]_D^{23}$ + 13.7° ($CHCl_3$) [lit.[15] $[\alpha]_D^{25}$ + 12.8° (neat)]. This acid **15** was treated with I_2 and $Pb(OAc)_4$ under irradiation with a tungsten lamp[18] to give an iodide **16**. Treatment of **16** with PhSNa yielded a phenyl sulfide **17**. This was oxidized with MCPBA to give a phenylsulfone (*S*)-**18**, $[\alpha]_D^{25}$ + 19.1° ($CHCl_3$), in 49.2% overall yield from **15**. The sulfone **18** was later attached to the steroid nucleus according to Kocienski's general method of olefin synthesis.[19,20] A synthesis of the antipode of this phenylsulfone, (*R*)-**18**, $[\alpha]_D^{40}$ − 12° ($CHCl_3$), had previously been reported by Kocienski *et al.*[20] Judging from the reported $[\alpha]_D$ value of (*R*)-**18**, their material seems to be optically impure.

Then the steroid-nucleus portion of brassinolide was prepared from the keto diol **7a**, an intermediate in our synthesis of (22*S*, 23*S*)-homobrassinolide (Scheme 3). Upon treatment with 2,2-dimethoxypropane and *p*-TsOH, the keto diol **7a** was converted to the corresponding acetonide **7b** in quantitative yield. After protecting the CO group as an ethylene acetal, **7c** was treated with O_3. Reductive work-up of the resulting ozonide with Me_2S in the presence of $NaHCO_3$ yielded a crystalline aldehyde **19** in 60% yield from **7b**.

With these two intermediates **18** and **19** in hand, we proceeded to the next stage. Addition of the aldehyde **19** to the carbanion derived from the sulfone **18** was followed by acetylation to give a β-acetoxy sulfone **20**. Reduction of **20** with Na–Hg in MeOH–EtOAc (2:1) gave an olefinic product **21a**, which upon deprotection furnished an unsaturated keto diol **21b** as crystals in 31% overall yield from **19**. This olefination reaction is known to give an (*E*)-olefin.[19,20] The (*E*)-geometry of the newly generated C-22 double bond of **21a** was supported by the 400 MHz ^{1}H-NMR spectral comparison of **21b** with **7a** ($J_{H\text{-}22,H\text{-}23}$ = 15.1 Hz for both **21b** and **7a**). Conventional acetylation of **21b** afforded the corresponding acetate **21c**.

For the introduction of the (22*R*, 23*R*)-glycol system to the olefin **21c**, a method other than OsO_4 oxidation had to be devised, since that oxidation yielded (22*S*, 23*S*)-glycol. After several model experiments with **7a** which resulted in the synthesis of (22*R*, 23*R*)-homobrassinolide,[21] we adopted the following procedure. The acetate **21c** was epoxidized with MCPBA to give a crystalline epoxide **22** in 82% yield as a stereoisomeric mixture. The epoxy ring in **22** was cleaved with 30% HBr in AcOH to give a bromo acetate by *trans*-opening of the epoxide. Another inversion at the carbon atom bearing the Br atom was effected by heating the bromo acetate with AcOH–H_2O (4:1). The product was acetylated with Ac_2O and DMAP in C_5H_5N to give the desired (22*R*, 23*R*)-tetraacetoxy ketone **23**, m.p. 221–224° (lit.[11] m.p. 215–217°) in 25.3% yield from **22** after chromatographic purification. This stereochemical outcome was the result of a double inversion at C-22 or C-23 of the (22*R*, 23*R*)-epoxide **22**. Another product of this reaction sequence was the stereoisomeric (22*S*, 23*S*)-tetraacetoxy ketone derived from the (22*S*, 23*S*)-epoxide **22**. This was a non-crystalline gum and readily removed from the desired ketone **23** by chromatography. The Baeyer–Villiger oxidation of **23** with CF_3CO_3H yielded brassinolide tetraacetate **1b**, m.p. 218–220°, in 82.9% yield. Hydrolysis of **1b** with NaOH was followed by acidification to give brassinolide **1a**, m.p. 273–274° (lit.[1] m.p. 274–275°; lit.[11] m.p. 273–278°; lit.[12] m.p. 273–274°). The IR, ^{1}H-NMR and ^{13}C-NMR spectra of our synthetic brassinolide were identical with the authentic spectra kindly supplied to us by Prof. N. Ikekawa.

In conclusion we were able to synthesize both brassinolide and (22*S*, 23*S*)-homobrassinolide in quantities sufficient for biological testings. Syntheses and biological activities of other analogs of brassinolide will be reported in due course.

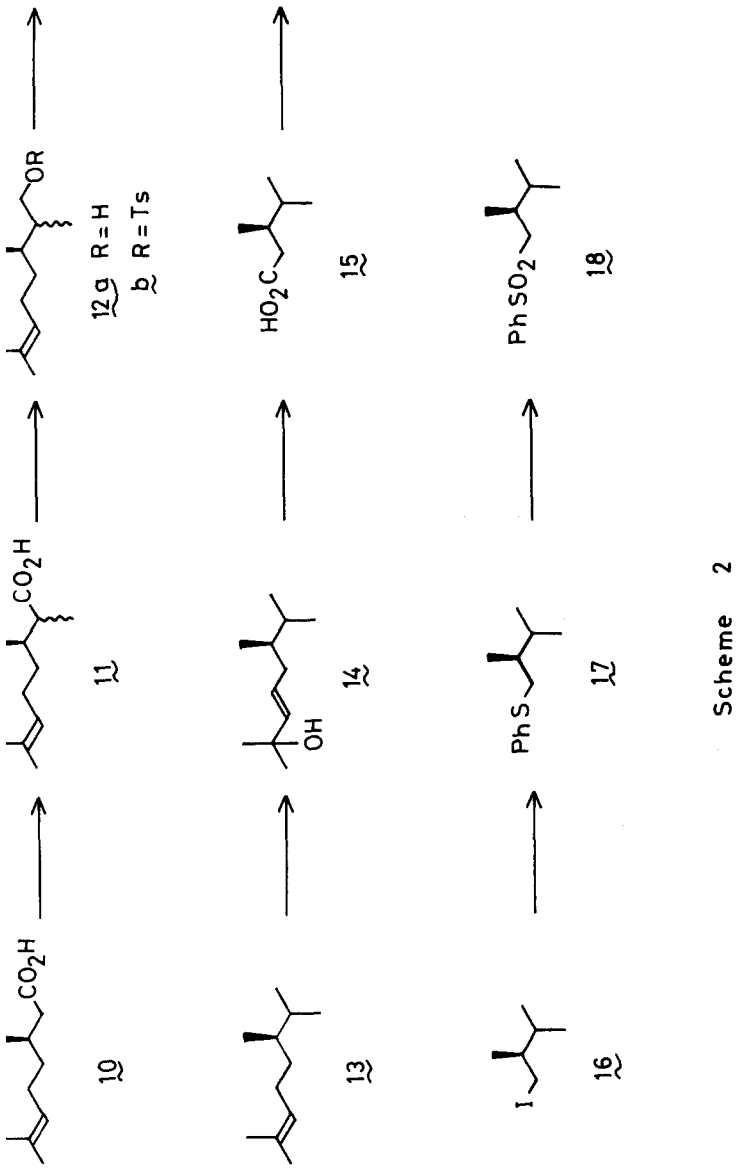
†This allylic alcohol **14** could also be prepared by a two-step procedure: (i) oxidation of **13** to the corresponding epoxide and (ii) cleavage of the epoxide with PhSeNa followed by H_2O_2-oxidation of the resulting phenylselenide (Experimental).

EXPERIMENTAL

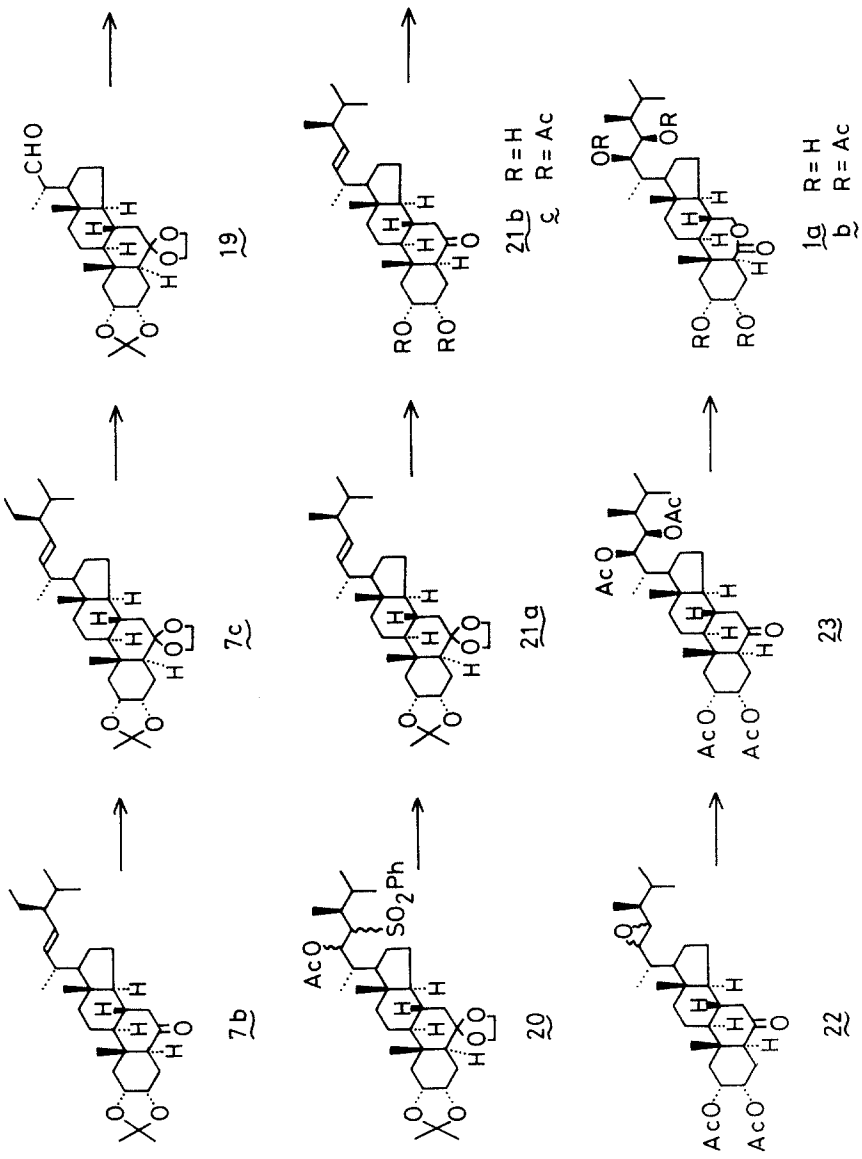
All b.ps and m.ps were uncorrected. IR spectra were determined as films for oils or as nujol mulls for solids on a Jasco A-102 spectrometer. NMR spectra were recorded at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer unless otherwise stated. Optical rotations were measured on a Jasco DIP-140 polarimeter. Hplc analyses were performed on a Shimadzu LC-2 chromatograph. Hplc separation was performed on a Waters System 500 apparatus.

24S-Ethyl-3α, 5-cyclo-5α-cholest-22E-en-6-one **5**. Jones 8N-CrO_3 (4 ml) was added dropwise to a stirred and ice-cooled soln of **4** (6.0 g) in acetone (100 ml). The mixture was stirred for 5 min. A small amount of MeOH was added to destroy the excess CrO_3. The mixture was concentrated *in vacuo*. The residue was diluted with water and extracted with ether. The ether soln was washed with water, $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was recrystallized from 99% EtOH to give 4.8 g (80%) of **5** as needles, m.p. 98–99°. Further recrystallization from 99% EtOH yielded an analytical sample, m.p. 102–103°, $[\alpha]_D^{21}$ + 19.5° (*c* = 8.000, $CHCl_3$); ν_{max} 1685 (s), 1310 (m), 1300 (s), 1250 (w), 1220 (w), 1200 (w), 1170 (m), 1155 (m), 1130 (m), 1120 (m), 1075 (w), 1055 (w), 1040 (w), 1020 (w), 1005 (w), 970 (s), 925 (w), 920 (w), 895 (w), 870 (w), 840 (w), 810 (w), 780 (w), 720 (w) cm^{-1}; δ ($CDCl_3$) 0.73, 0.80, 0.86, 0.90, 1.02, 1.10 (18 H CH_3), 1.1 2.7 (26H, CH_2, CH), 5.15 (2H, –CH=). (Found: C, 85.41: H, 11.44. Calc. for $C_{29}H_{46}O$: C, 84.81; H, 11.29%).

24S-Ethyl-5α-cholesta-2, 22E-dien-6-one **6**. *p*-TsOH (180 mg) was added to a suspension of **5** (3.5 g) in sulfolane (25 ml) and the mixture was heated at 160° for 70 min. After cooling, the mixture was poured into water and extracted with C_6H_6-ether (1:1). The extract was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (Merck Kieselgel 60, Art 7734, 35 g in *n*-hexane). Elution with *n*-hexane-C_6H_6 (1:1) gave 2.3 g (66%) of **6**. This was recrystallized from 99% EtOH to give prisms, m.p. 111–112°, $[\alpha]_D^{21}$ + 18.4° (*c* = 1.151, $CHCl_3$): ν_{max} 3020 (m), 1705 (s), 1650 (w), 1330 (w), 1310 (w), 1300 (w), 1285 (w), 1260 (m), 1230 (m), 1190 (w), 1180 (w), 1160 (w), 1120 (w), 1100 (w), 1070 (m), 1045 (w), 1020 (w), 995 (m), 965 (s), 940 (w), 925 (w), 870 (w), 845 (w), 815 (w), 780 (w), 750 (w), 720 (w), 670 (m) cm^{-1}; δ ($CDCl_3$) 0.69, 0.78, 0.84, 0.88, 0.96, 1.08 (18 H, CH_3), ~1.1 – ~2.5 (24H, –CH_2, –CH),


CO2H
10
CO2H
11
OR
12a R=H
b R=Ts
OH
13
OH
14
HO2C
15
I
16
PhS
17
PhSO2
18

Scheme 2


CHO
19
7c
21b R=H
c R=Ac
21a
1g R=H
b R=Ac
7b
20
22
23
AcO
SO2Ph
OAc
OR
RO
Scheme 3

~5.10 (2 H), 5.60 (2 H, br. s). (Found C, 85.19; H, 11.60. Calc. for $C_{29}H_{46}O$: C, 84.81; H, 11.29%).

2α, 3α-*Dihydroxy-24S-ethyl-5α-cholest-22E-en-6-one* **7a**. A soln of OsO_4 (300 mg) in *t*-BuOH (10 ml) was added to a soln of **6** (6.0 g) in acetone (300 ml). Then NMO (6.0 g) and H_2O (10 ml) were added to it. The mixture was stirred under Ar for 10 hr. The precipitated crystals were collected on a filter. The filtrate was concentrated *in vacuo*. After destroying the excess OsO_4 with $NaHSO_3$ aq, the mixture was extracted with $CHCl_3$. The extract was washed with dil HCl and brine, dried (K_2CO_3), decolorized with activated charcoal and concentrated *in vacuo*. The residue was triturated with ether-acetone to give **7a**. The combined product **7a** was recrystallized from 99% EtOH to give 4.1 g (63%) of **7a** as needles m.p. 235–238° (dec), $[\alpha]_D^{21}$ −9.2° (*c* = 1.071, $CHCl_3$); ν_{max} ~3360 (m), 1715 (s), 1700 (sh), 1330 (w), 1310 (w), 1290 (w), 1265 (w), 1240 (w), 1210 (w), 1165 (w), 1150 (w), 1120 (w), 1105 (w), 1080 (m), 1055 (m), 1040 (m), 1015 (w), 990 (w), 970 (m), 940 (w), 930 (w), 875 (w), 725 (w) cm^{-1}; δ (400.5 MHz, $CDCl_3$) 0.68 (3 H, s), 0.76 (3 H, s), 0.79 (3 H, d, J = 6.4 Hz), 0.80 (3H, t, J = 7.3 Hz), 0.84 (3H, d, J = 6.4 Hz), 1.02 (3H, d, J = 6.4 Hz), 1.18–2.02 (m), 2.29 (1H, dd, J = 4.4 and 13.2 Hz), 2.68 (1H, dd, J = 3.2 and 12.5 Hz), 3.76 (1H, ddd, J = 3.3, 5.0 and 11.7 Hz), 4.05 (1H, dt, J = 2.8 and 3.3 Hz), 5.02 (1H, dd, J = 8.3 and 15.1 Hz), 5.14 (1H, dd, J = 8.8 and 15.1 Hz). (Found: C, 77.81; H, 10.92. Calc. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88%). In one occasion **7a** was obtained in 97.8% yield from **6**.

2α, 3α, 22*S*, 23*S*-*Tetrahydroxy-24S-ethyl-5α-cholestan-6-one* **8a**. A soln of OsO_4 (210 mg) in *t*-BuOH (7 ml) was added to a soln of **6** (4.0 g) in THF (80 ml). Then NMO (3 g) and water (20 ml) were added to it. The mixture was stirred for 4 days at room temp. under Ar. Additional amount of NMO (1 g × 2) was added during that period. Then excess OsO_4 was reduced with $Na_2S_2O_4$ soln. The mixture was filtered through Celite. The filtrate was concentrated *in vacuo* and extracted with $CHCl_3$. The extract was washed with dil HCl, dried (K_2CO_3) and concentrated *in vacuo*. The residue was triturated with ether–pet. ether to give 2.4 g (52%) of **8a**. From the mother liquor 1.5 g of impure **8a** was obtained. The crude **8a** was recrystallized from 99% EtOH to give prisms, m.p. 206–208°, $[\alpha]_D^{21}$ −1.9° (*c* = 1.294 $CHCl_3$); ν_{max} ~3350 (s), 1715 (s), 1340 (m), 1320 (m), 1310 (m), 1280 (m), 1250 (m), 1240 (m), 1230 (m), 1205 (w), 1180 (w), 1155 (w), 1115 (m), 1100 (m), 1080 (s), 1065 (s), 1055 (s), 1050 (s), 1035 (s), 1010 (s), 1000 (m), 990 (m), 965 (w), 940 (w), 930 (w), 900 (w), 870 (w), 840 (w), 790 (w), 780 (w), 750 (w), 720 (w), 700 (w) cm^{-1}. (Found: C, 73.03; H, 11.17. Calc. for $C_{29}H_{50}O_5$: C, 72.76: H, 10.53%).

2α, 3α, 22S, 23S-bis-*Isopropylidenedioxy-24S-ethyl-5α-cholestan-6-one* **8b**

(a) *Small-scale preparation from* **7a**. OsO_4 (63.5 mg, 0.25 m mol) and **7a** (111 mg, 0.25 m mol) were dissolved in dry C_5H_5N (1.0 ml) and the mixture was stirred for 24 hr in a dark place. Then $NaHSO_3$ (120 mg) in C_5H_5N (1.3 ml)-H_2O (1.9 ml) was added to the mixture. After stirring for 30 min at room temp, the mixture was extracted with $CHCl_3$. The extract was washed with water, dil HCl, water and $NaHCO_3$ soln, dried (Na_2SO_4) and concentrated *in vacuo* to give a dark greenish syrup (174 mg). This was dissolved in CH_2Cl_2 (5 ml)-C_6H_6 (5 ml). To this were added 2,2-dimethoxypropane (3 ml) and *p*-TsOH (0.1 g). The mixture was stirred for 17 hr at room temp. Then K_2CO_3 (1 g) was added and the stirring was continued for 5 min. The mixture was washed with $NaHCO_3$ aq, dried (K_2CO_3) and concentrated *in vacuo* to give 198 mg of syrup. This was analyzed by hplc (Partisil-5, 25 cm × 4.6 mm; Elution with *n*-hexane–EtOAc (5 : 1); flow rate : 2 ml/min). Three peaks were observed: Peak 1 (R_t 13.0 min) corresponded to **7b** (8.9%); Peak 2 (R_t 16.0 min) corresponded to (22*R*, 23*R*)-isomer of **8b** (2.5%); Peak 3 (R_t 16.5 min) corresponded to **8b** (88.6%). The syrup was chromatographed over SiO_2 (Merck Kieselgel Art 7734, 12 g). Elution with *n*-hexane–EtOAc (10 : 1) yielded pure crystalline **8b** (84 mg, 60% from **7a**) as needles, m.p. 185–186°; $[\alpha]_D^{22.5}$ +11.6° (*c* = 0.110, $CHCl_3$); ν_{max} 1705 (s), 1455 (m), 1450 (m), 1370 (m), 1360 (m), 1330 (w), 1290 (w), 1235 (s), 1210 (s), 1160 (m), 1150 (w), 1120 (w), 1090 (w), 1080 (w), 1045 (s), 1010 (w), 980 (w), 960 (w), 910 (w), 880 (w), 840 (w), 820 (w), 780 (w) cm^{-1}; δ ($CDCl_3$) 0.68 (3H, s), 0.8–2.6 (m), 1.23 (3H, s), 1.35 (9H, s), 1.49 (3H, s), 3.5–4.4 (4H, m); MS: *m/z* 558 (M^+), 557 (M^+–H), 544, 543 (M^+–Me), 473 (M^+–EtCH-CHMe$_2$), 429, 370. (Found: C, 74.61; H, 10.38. Calc. for $C_{35}H_{58}O_5$: C, 75.22; H, 10.46%). Upon hplc analysis this pure **8b** showed a single peak at R_t 16.8 min under the same condition as those used for the analysis of the crude syrup.

(b) *Large-scale preparation from* **8a**. Three drops of conc H_2SO_4 was added to a soln of **8a** (7.5 g) in 2,2-dimethoxypropane (50 ml) and the mixture was stirred overnight at room temp. Then it was poured into K_2CO_3 soln and extracted with ether. The ether soln was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed over Al_2O_3 (Woelm neutral Al_2O_3, grade II) to give 7.5 g (72%) of crude **8b**. This was purified by preparative hplc (Waters Prep LC/System 500A, Prep PAK-SILICA; flow rate 100 ml/min, *n*-hexane-THF = 10 : 1). The desired **8b** was eluted at R_t = 13.2 min as 5.9 g of needles. Recrystallization of the crude **8b** from *n*-hexane–ether gave pure **8b**, m.p. 185–186°, δ (^{13}C-NMR, $CDCl_3$) 72.1, 72.3, 77.4, 79.8, 106.7, 107.8, 211. (Found: C, 74.67; H, 10.48. Calc. for $C_{35}H_{58}O_5$: C, 75.22; H, 10.46%).

2α, 3α, 22S, 23S-*Tetraacetoxy-24S-ethyl-5α-cholestan-6-one* **8c**. Ac_2O (1 ml) and DMAP (0.1 g) were added to a soln of **8a** (380 mg) in dry C_5H_5N (3 ml). The mixture was left to stand overnight at room temp. Then it was poured into ice-dil HCl and extracted with ether. The ether soln was washed with water, $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (Merck Kieselgel 60, Art 7734, 5 × 1.5 cm in *n*-hexane). Elution with *n*-hexane–EtOAc (9 : 1 ~ 4 : 1) yielded 500 mg (95%) of **8c** as a gum, ν_{max} 1740 (s), 1705 (s), 1230 (s), 1170 (m), 1150 (m), 1100 (w), 1070 (w), 1030 (m), 1010 (m), 980 (w), 965 (w), 940 (w), 915 (w), 890 (w), 875 (w) cm^{-1}. This was employed for the next step without further purification.

2α, 3α, 22S, 23S-*Tetraacetoxy-24S-ethyl-B-homo-7-oxa-5α-cholestan-6-one* [= (22S, 23S)-*homobrassinolide tetraacetate*] **2b**. A soln of CF_3CO_3H in CH_2Cl_2 was prepared by careful addition of $(CF_3CO)_2O$ (3.3 ml) into an ice-cooled mixture of 90% H_2O_2 (0.5 ml) and CH_2Cl_2 (5 ml) with shaking. The resulting homogeneous soln of the peracid was added dropwise to a stirred mixture of **8c** (598 mg) and finely powdered Na_2HPO_4 (3 g) in CH_2Cl_2 (30 ml). When the exothermic reaction subsided, the mixture was stirred and heated under reflux for 1.5 hr. Then it was poured into ice-water and extracted with CH_2Cl_2. The CH_2Cl_2 soln was washed with $NaHCO_3$ aq, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed over SiO_2 (Mallinckrodt Silicar CC-7, 20 × 1.6 cm in *n*-hexane). Elution with *n*-hexane–EtOAc (9 : 1 ~ 4 : 1) yielded 405 mg (60%) of **2b**, m.p. 174–178°. In a large-scale preparation, 5.6 g of **8c** yielded 5.6 g of **2b**. An analytical sample was obtained as needles when recrystallized from EtOAc-pet. ether, m.p. 185–187°, $[\alpha]_D^{20}$ +24.4° (*c* = 1.006, $CHCl_3$); ν_{max} 1740 (s), 1720 (s), 1330 (w), 1315 (w), 1305 (w), 1250 (s), 1225 (s), 1180 (m), 1170 (w), 1125 (w), 1115 (w), 1050 (sh), 1040 (m), 1015 (m), 960 (w), 940 (w), 915 (w), 900 (w), 890 (w), 875 (w), 765 (w), 720 (w) cm^{-1}; δ ($CDCl_3$) ~0.6 – ~1.1 (18H, CH_3, 0.70, 0.79, 0.89, 0.98), 2.00 (3H, s), 2.07 (3H, s), 2.10 (6H, s), ~3.0 (1H, m, CHCO), 4.10 (2H, d, J = 7 Hz, –CH_2O), ~4.70 – ~5.50 (4H, –CHOAc, ~4.8, ~5.0, ~5.2, 5.35). (Found: C, 66.93; H, 8.86. Calc. for $C_{37}H_{58}O_{10}$: C, 67.04; H, 8.82%).

2α, 3α, 22S, 23S-*Tetrahydroxy-24S-ethyl-B-homo-7-oxa-5α-cholestan-6-one* [= (22S, 23S)-*homobrassinolide*] **2a**. NaOH soln (500 mg in 1 ml H_2O) was added to a soln of **2b** (250 mg) in MeOH (6 ml) and the mixture was stirred and heated under reflux for 1 hr. The stirring was continued for 1 hr at room temp. The mixture was diluted with THF (6 ml), acidified with 6N–HCl (3 ml) and stirred and heated under reflux for 30 min. Then it was concentrated *in vacuo*. The residual aq soln was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried ($MgSO_4$) and concentrated *in vacuo* to give 130 mg (70%) of **2a**, m.p. 186–188°. This was recrystallized from MeOH to give needles, m.p. 193–194°, $[\alpha]_D^{20}$ +35.6° (*c* = 0.54, $CHCl_3$); ν_{max} ~3400 (br. s), 1730 (sh), 1710 (sh), 1695 (s), 1405 (m), 1350 (m), 1330 (m), 1290 (sh), 1280 (m), 1270 (sh), 1250 (w), 1225 (sh), 1220 (m), 1190 (m), 1180 (m), 1160 (w), 1130 (m), 1120 (m), 1100 (w), 1065 (s), 1020 (m), 990 (m), 965 (w), 950 (w), 930 (w), 915 (w), 880 (w),

860 (w), 835 (w), 820 (w), 770 (w), 715 (w) cm^{-1}; δ ($CDCl_3$) ~ 0.6– ~ 1.1 (~ 18H, $-CH_3$, 0.70, 0.78, 0.87, 0.95), ~ 1.1 – ~ 2.5 (~ 22H, $-CH_2$, -CH), ~ 2.90 (~ 2H), 3.55 (~ 2H), ~ 4.0 (2H, br. $-CH_2O$); δ (^{13}C-NMR, $CDCl_3$) 67.9, 68.1, 70.4, 70.9, 72.1 (C–O), 176.9 (C=O). (Found: C, 70.71; H, 10.29. Calcd. for $C_{29}H_{50}O_6$: C, 70.41; H, 10.19%).

(2RS, 3R)-2, 3, 7-*Trimethyl-6-octenoic acid* **11**. A soln of LDA was prepared from (i-Pr)$_2$NH (119 ml) and 1.5 N-*n*-BuLi (573 ml) in THF (300 ml) at −20° under Ar. A soln of **10** [33.6 g, $[\alpha]_D^{24}$ + 10.5° (c = 2.08, $CHCl_3$)] in THF (200 ml) was added dropwise with stirring at −20°. Then HMPA (50 ml) was added at −5° and the mixture was stirred at −5 ~ 0° for 30 min. Then it was cooled to −30° and MeI (150 g) was added during 15 min. At the end of the addition the temp rose to 10°. The mixture was stirred for 2 hr at 10–20°, diluted with water and concentrated. The residue was diluted with water and extracted with ether (X2) to remove neutral impurities. The aq soln was acidified with dil HCl and extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated. The residue was distilled to give 28.3 g (78%) of **11**, b.p. 115–128°/0.2 mm, n_D^{24} 1.4521; $[\alpha]_D^{24}$ + 13.8° (c = 1.02, $CHCl_3$); ν_{max} ~ 3600–2400 (m), 1710 (s), 1450 (m), 1410 (m), 1380 (m), 1280 (m), 1230 (m), 1120 (w), 1070 (w), 940 (m), 860 (w), 830 (w), 730 (w) cm^{-1}; δ ($CDCl_3$) 0.60–2.70 (12H, m), 1.60 (3H, s), 1.68 (3H, s), 5.01 (1H, t, J = 7 Hz), 10.95 (1H, br. s). (Found: C, 71.13; H, 10.95. Calc. for $C_{11}H_{20}O_2$: C, 71.69; H, 10.94%).

(2RS, 3R)-2, 3, 7-*Trimethyl-6-octen-1-ol* **12a**. A soln of **11** (18.8 g) in dry ether (70 ml) was added dropwise to a stirred and ice-cooled slurry of LAH (5.63 g) in dry ether (530 ml). The mixture was stirred at toom temp overnight. The excess LAH was destroyed by successive addition of H_2O (6 ml), 10% NaOH soln (6 ml) and H_2O (12 ml) under ice-cooling. The stirring was continued for 30 min and the mixture was filtered. The filtrate was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 16.3 g (94%) of **12a**, b.p. 75–78°/0.4 mm, n_D^{23} 1.4553; $[\alpha]_D^{23}$ + 17.5° (c = 1.25, $CHCl_3$); ν_{max} 3350 (s), 1450 (m), 1380 (m), 1030 (s), 820 (w) cm^{-1}; δ (CCl_4) 0.78 (3H, d, J = 6 Hz), 0.88 (3H, d, J = 6 Hz), 1.58 (3H, s), 1.66 (3H, s), 3.3–3.8 (3H, m), 5.05 (1H, t, J = 6 Hz). (Found: C, 77.54; H, 13.28. Calc. for $C_{11}H_{22}O$: C, 77.58; H, 13.02%).

(2RS, 3R)-2, 3, 7-*Trimethyl-6-octen-1-ol tosylate* **12b**. Powdered *p*-TsCl (20.0 g) was added portionwise to a stirred and ice-cooled soln of **12a** (16.2 g) in dry C_5H_5N (70 ml) and the mixture was left to stand overnight. Then it was poured into ice-water and extracted with ether. The ether extract was washed with water, $CuSO_4$ aq, $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give crude **12b** (30.8 g, quantitative), ν_{max} 1600 (m), 1490 (w), 1450 (m), 1360 (s), 1300 (w), 1290 (w), 1210 (w), 1190 (s), 1180 (s), 1110 (w), 1100 (m), 1040 (w), 1020 (w), 960 (s), 840 (s), 810 (s), 770 (m), 700 (w), 680 (w), 660 (s) cm^{-1}; δ (CCl_4) 0.6–0.9 (6H, m), 1.52 (3H, s), 1.61 (3H, s), 2.38 (3H, s), 3.55–3.95 (2H, m), 4.98 (1H, t, J = 7 Hz), 7.25 (2H, d, J = 7 Hz), 7.66 (2H, d, J = 7 Hz). This was employed for the next step without further purification.

(R)-2, 6, 7-*Trimethyl-2-octene* **13**. A soln of crude **12b** (30.8 g) in dry ether (50 ml) was added dropwise to a stirred and ice-cooled slurry of LAH (7.2 g) in dry ether (500 ml). After stirring for 5 hr at room temp, the excess LAH was decomposed by careful addition of water (35 ml) under ice-cooling. Then the mixture was poured into ice-dil HCl. The ether layer was separated and the aq layer was extracted with ether. The combined ether soln was washed with water, $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated. The residue was distilled to give 12.6 g (86% from **12a**) of **13**, b.p. 90–91°/42 mm, n_D^{23} 1.4326; $[\alpha]_D^{23}$ + 22.2° (c = 1.38, $CHCl_3$); ν_{max} 1450 (m), 1370 (m), 1110 (w), 1090 (w), 1020 (w), 980 (w), 820 (w) cm^{-1}; δ (CCl_4) 0.76 (6H, d, J = 6 Hz) 0.85 (3H, d, J = 6 Hz), 1.55 (3H, s), 1.64 (3H, s), 5.03 (1H, t, J = 7 Hz). (Found: C, 85.39; H, 14.27 Calc. for $C_{11}H_{22}$: C, 85.63; H, 14.37%).

(R)-2, 6, 7-*Trimethyl-3-octen-2-ol* **14**. To a stirred and ice-cooled soln of Ph_2Se_2 (25.74 g) in dry CH_2Cl_2 (280 ml) was slowly added chilled 35% H_2O_2 (8.01 g). After stirring vigorously for 30 min (white crystals deposit in 5–10 min), powdered $MgSO_4$ (13.78 g) was added and the mixture was stirred for an additional 30 min in the ice-bath. Then, **13** (8.48 g) was added, and the mixture was stirred vigorously for 6 hr at room temp. Chilled 70% *t*-BuOOH (40.3 ml) was added to the mixture with ice-cooling. After removing the ice-bath, the mixture was stirred for 20 hr at room temp to give a pale orange soln with a lot of white ppt. This ppt was filtered off and washed with ether. The filtrate was concentrated to give an oil. The oil was dissolved in ether (350 ml) and washed with 5% Na_2CO_3 aq (200 ml × 2), water, 10% $FeSO_4$ aq (200 ml × 2), water, $NaHCO_3$ aq, water and brine, successively. The ether soln was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed over SiO_2. Thus obtained **14** was distilled to give 6.32 g (67.5%) of pure **14**, b.p. 54–56°/0.4 mm, n_D^{22} 1.4434; $[\alpha]_D^{22}$ + 4.61° (c = 1.91, $CHCl_3$), ν_{max} 3370 (m), 1460 (m), 1380 (m), 1370 (m), 1230 (w), 1150 (m), 1110 (m), 1050 (w), 1020 (w), 970 (m), 920 (w), 890 (w), 780 (w) cm^{-1}; δ (CCl_4) 0.65–1.00 (9H, m), 1.20 (6H, s), 2.65 (1H, s, -OH), 5.38–5.61 (2H, m). (Found: C, 77.75; H, 12.98. Calc. for $C_{11}H_{22}O$: C, 77.58; H, 13.07%).

(3RS, 6R)-2, 3-*Epoxy*-2, 6, 7-*trimethyloctane*. MCPBA (80%, 46 g) was added portion-wise to a stirred and ice-cooled solution of **13** (33.3 g) in CH_2Cl_2 (400 ml). After the addition the mixture was stirred overnight at 0–5°. The precipitated MCBA was collected on a filter and washed with *n*-hexane. The combined filtrate and washings were washed with 10% $NaHSO_3$ aq and 20% Na_2CO_3 aq, dried (K_2CO_3) and concentrated. The residue was distilled to give 31 g (84%) of the epoxide, b.p. 107–108°/39 mm, n_D^{24} 1.4232; $[\alpha]_D^{24.5}$ + 16.8° (c = 1.46, $CHCl_3$); ν_{max} 1460 (m), 1380 (s), 1120 (m) cm^{-1}; δ (CCl_4) 0.75–0.95 (9H, m), 1.15 (3H, s), 1.20 (3H, s), 1.25–1.70 (6H, m), 2.50 (1H, t, J = 4 Hz). (Found: C, 77.15; H, 12.91. Calc. for $C_{11}H_{22}O$: C, 77.58; H, 13.02%).

(R)-2, 6, 7-*Trimethyl-3-octen-2-ol* **14** *from the epoxide*.[22] A soln of PhSeNa in EtOH was prepared by the addition of $NaBH_4$ (7.7 g) in small portions to a stirred and ice-cooled suspension of Ph_2Se_2 (32 g) in EtOH (400 ml) under N_2. The above described epoxide (31 g) was added dropwise to the resulting clear soln of PhSeNa. After the addition, the mixture was stirred and heated under reflux for 2 hr. After cooling, the mixture was diluted with THF (250 ml). Then 35% H_2O_2 (200 ml) was added dropwise to the stirred and ice-cooled mixture keeping the reaction temp below 20°. The stirring was continued for 2 hr. The mixture was diluted with water (500 ml) and extracted with ether. The ether soln was washed with Na_2CO_3 aq, dried (Na_2SO_4) and concentrated. The residue was distilled to give 27 g (87%) of **14**, b.p. 92–93°/13 mm. The spectral data were identical with those prepared from 13 in a single step. The overall yield of **14** from **13** by this two-step procedure was 73% and slightly better than the single-step procedure. Although the single step procedure was more convenient than the two-step method, it was less reproducible in our hands.

(*R*)-3, 4-*Dimethylpentanoic acid* **15**. O_3 was bubbled into a soln of **14** (6.71 g) in MeOH (400 ml) with stirring at −65 ~ −70° for 1.5 hr. Then Me_2S (20 ml) was added and the mixture was stirred overnight at room temp. Subsequently it was concentrated and diluted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated to give a crude oil (4.15 g). Its IR[ν_{max} 2700 (w), 1730 (m), 1120 (s), 1000 (s)] and NMR[δ 3.13 (s, $-OCH_3$), 4.28 (t, $CH\langle^{OMe}_{OMe}$), 9.57 (s, CHO)] revealed it to be a mixture of (*R*)-3, 4-dimethylpentanal and its dimethyl acetal. This crude mixture was stirred with 35% $HClO_4$-THF (1 : 1, 20 ml) for 20 min at room temp. The mixture was carefully neutralized with powdered $NaHCO_3$ and extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 3.2 g of an oil, ν_{max} 2700 (w), 1730 (s), 1470 (m), 1380 (m), 1110 (m), 1040 (m), 960 (w) cm^{-1}. Jones CrO_3 reagent (8N, 7 ml) was added to a stirred and ice-cooled soln of the oil (3.2 g) in acetone (30 ml). The mixture was stirred for 30 min at room temp. MeOH was added to destroy the excess CrO_3. The mixture was concentrated *in vacuo*. The residue was diluted with water and extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 2.56 g (50% from **14**) of **15** as an oil, b.p. 87–90°/1.8 mm, $n_D^{23.5}$ 1.4220; $[\alpha]_D^{23.5}$ + 13.7° (c = 1.54, $CHCl_3$); ν_{max} 3700–2200 (m), 1710 (s), 1460 (m), 1410 (m), 1390 (m), 1370 (m), 1290 (m), 1210 (m), 1150 (w), 1110 (m), 940 (m) cm^{-1}; δ 0.7–1.1 (9H, m), 1.2–2.5 (4H, m), 11.95 (1H, s). (Found: C, 64.77; H, 11.03. Calc. for $C_7H_{14}O_2$: C, 64.58; H, 10.84%).

(S)-2, 3-*Dimethylbutyl iodide* **16**. The acid **15** (1.63 g) was heated

under reflux in dry CCl_4 (185 ml) containing $Pb(OAc)_4$ (7.64 g) and the soln was irradiated with a tungsten filament lamp while I_2 (2.80 g) was added in small portions at intervals; decolorization was allowed to take place before each further addition. Finally the coloration persisted. Heating was continued for a further 30 min, and the soln was cooled, filtered through Celite, washed with $Na_2S_2O_3$ soln, and then dried ($MgSO_4$) and concentrated. The resulting crude **16** (2.60 g) was employed for the next step without further purification, ν_{max} 1460 (m), 1420 (w), 1390 (m), 1380 (m), 1370 (m), 1290 (w), 1270 (w), 1240 (w), 1190 (m), 1180 (m), 1020 (w), 880 (w), 790 (s) cm^{-1}; δ (CCl_4) 0.5–2.2 (11H, m), 2.7–3.5 (2H, m).

(S)-2, 3-*Dimethylbutyl phenyl sulfide* **17**. PhSH (1.5 g) was added to a soln of NaOEt (from 0.33 g of Na) in EtOH (38 ml). To this was added **16** (2.60 g). The mixture was stirred for 2.5 hr at room temp. Then it was diluted with water (160 ml) and extracted with pet. ether (50 ml × 2). The extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed over SiO_2. The resulting **17** was distilled to give 2.00 g (82% from **15**) of pure **17**, b.p. 115–117°/1.2 mm, $n_D^{23.5}$ 1.5302; $[\alpha]_D^{23.5}$ + 45.8° (c = 1.73, $CHCl_3$); ν_{max} 3070 (w), 1590 (m), 1480 (s), 1460 (m), 1440 (s), 1390 (m), 1380 (m), 1300 (w), 1240 (w), 1150 (w), 1110 (w), 1090 (m), 1070 (w), 1030 (m), 740 (s), 690 (s) cm^{-1}; δ 0.70–1.15 (9H, m), 1.15–2.05 (2H, m), 2.57 (1H, dd, J = 7.5 and 12 Hz), 2.93 (1H, dd, J = 5 and 12 Hz), 6.90–7.36 (5H, m). (Found: C, 74.37; H, 9.27. Calc. for $C_{12}H_{18}S$: C, 74.16; H, 9.34%).

(S)-2, 3-*Dimethylbutyl phenyl sulfone* **18**. To a stirred soln of **17** (2.59 g) in CH_2Cl_2 (100 ml), MCPBA (85%, 6.00 g) was added portionwise and the mixture was stirred for 1.5 hr at room temp. Then it was washed with NH_3 soln and water, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was further purified by chromatography over SiO_2 to give 2.40 g (80%) of **18**. An analytical sample boiled at 116°/0.5 mm, n_D^{23} 1.5141; $[\alpha]_D^{23}$ + 19.1° (c = 1.69, $CHCl_3$); ν_{max} 3060 (w), 1590 (w), 1460 (m), 1450 (m), 1400 (w), 1390 (w), 1370 (w), 1300 (s), 1240 (w), 1150 (s), 1090 (s), 1070 (w), 1020 (w), 1000 (w), 840 (w), 790 (w), 770 (w), 740 (s), 710 (w), 690 (s) cm^{-1}; δ (CCl_4) 0.74 (3H, d, J = 6 Hz), 0.79 (3H, d, J = 6 Hz), 0.97 (3H, d, J = 6 Hz), 1.32–2.32 (2H, m), 2.67 (1H, dd, J = 7 and 13 Hz), 2.99 (1H, dd, J = 4 and 13 Hz), 7.30–8.02 (5H, m). (Found: C, 63.64; H, 8.32. Calc. for $C_{12}H_{18}O_2S$: C, 63.68; H, 8.02%).

2α, 3α-*Isopropylidenedioxy*-24S-*ethyl*-5α-*cholest*-22E-*en*-6-*one* **7b**. A great excess of 2,2-dimethoxypropane (40 ml) and *p*-TsOH (1.0 g) were added to a soln of **7a** (20 g) in CH_2Cl_2 (500 ml). The mixture was stirred for 1 hr at room temp. After the addition of K_2CO_3 powder, the mixture was stirred for 5 min. Then it was washed with $NaHCO_3$ aq, dried (K_2CO_3) and concentrated *in vacuo* to give 23 g (quantitative) of **7b**. An analytical sample was recrystallized from 99% EtOH to give needles, m.p. 158–159°, $[\alpha]_D^{23}$ + 21.1° (c = 0.645, $CHCl_3$); ν_{max} 1700 (sh), 1690 (s), 1458 (s), 1445 (s), 1373 (s), 1365 (s), 1290 (w), 1235 (m), 1210 (m), 1050 (s), 1035 (w), 1015 (w), 965 (w), 840 (w), 780 (w) cm^{-1}; δ ($CDCl_3$) 0.6–2.8 (m, singlets at 0.68, 1.33, 1.50), 3.81–4.36 (2H, m), 4.90–5.21 (2H, m). (Found: C, 79.58; H, 10.83. Calc. for $C_{32}H_{52}O_3$: C, 79.28; H, 10.81%).

6, 6-*Ethylenedioxy*-2α, 3α-*isopropylidenedioxy*-24S-*ethyl*-5α-*cholest*-22E-*ene* **7c**. *p*-TsOH (0.3 g) was added to a soln of **7b** (5.2 g) in butanone ethyleneacetal (50 ml) and the soln was stirred and heated under reflux for 3 hr. The mixture was neutralized by the addition of K_2CO_3 (2 g) and diluted with ether (200 ml). The ether soln was washed with $NaHCO_3$ aq, dried (K_2CO_3) and concentrated *in vacuo* to give 5.2 g of gummy **7c**, ν_{max} 2950 (s), 2870 (s), 1460 (m), 1372 (m), 1330 (w), 1300 (w), 1285 (w), 1250 (w), 1222 (w), 1190 (m), 1167 (m), 1143 (m), 1125 (m), 1061 (s), 1042 (s), 972 (m), 945 (m), 915 (w), 890 (w), 861 (m), 835 (w), 775 (w), 735 (w), 705 (w) cm^{-1}. This was employed for the next step without purification.

6, 6-*Ethylenedioxy*-2α, 3α-*isopropylidenedioxy*-5α-*pregnane*-20S-*carboxaldehyde* **19**. O_3 was bubbled into a soln of **7c** (5.2 g) in CH_2Cl_2 (600 ml) and MeOH (600 ml) in the presence of $NaHCO_3$ (5 g) at −60 ~ −50°. After the soln was saturated with O_3, excess O_3 was bubbled for further 3 hr. Then the mixture was left to stand at −60 ~ −50° for 1 hr and the excess O_3 was driven out by bubbling N_2. Me_2S (30 ml) was added to the stirred mixture. After 3 hr the cooling bath was removed and the stirring was continued overnight at room temp. The mixture was diluted with ether (2 l). The ether soln was washed throughly with water (500 ml × 8), dried (Na_2SO_4) and concentrated *in vacuo*. The residue (5.0 g) was chromatographed over SiO_2 (Mallinckrodt Silicar CC-7, 200 g). Elution with *n*-hexane–EtOAc (9 : 1 ~ 8 : 1) yielded 2.87 g (60%) of **19**, which crystallized after standing in a refrigerator, m.p. 118–121°, $[\alpha]_D^{24.5}$ + 38.1° (c = 1.264, $CHCl_3$); ν_{max} 2700 (w), 1730 (s), 1460 (s), 1450 (m), 1375 (m), 1335 (w), 1305 (w), 1285 (w), 1280 (w), 1250 (w), 1230 (w), 1215 (w), 1195 (m), 1165 (s), 1130 (m), 1125 (m), 1085 (m), 1060 (s), 1045 (m), 1040 (m), 1010 (w), 1000 (w), 980 (w), 960 (w), 945 (w), 940 (w), 920 (w), 885 (w), 865 (w), 850 (w), 840 (w) cm^{-1}; δ ($CDCl_3$) 0.6–2.6 (m, singlets at 0.71 and 0.83), 3.5–4.3 (6H, m), 9.47 (1H, d, J = 3 Hz); MS: m/z 446 (M^+), 431 (M^+–Me), 389 (M^+–MeCH-CHO). (Found: C, 71.68:; H, 9.58. Calc. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48%).

6, 6-*Ethylenedioxy*-2α, 3α-*isopropylidenedioxy*-22ξ-*acetoxy*-23ξ-*phenylsulfonyl*-24S-*methyl*-5α-*cholestane* **20**. *n*-BuLi in *n*-hexane (1.7 N, 1.72 ml; 2.92 m mol) was added to a stirred and cooled soln of **18** (660 mg; 2.92 m mol) in dry THF (30 ml) at −70 ~ −60° under Ar. After stirring for 30 min, a soln of **19** (1.136 g, 2.57 m mol) in dry THF (10 ml) was added. After stirring for 1 hr, Ac_2O (0.57 ml; 6.0 m mol) was added. The reaction temp was gradually raised to room temp during 2 hr and the stirring was continued overnight at room temp. The mixture was poured into ice-$NaHCO_3$ aq and extracted with ether. The ether soln was washed with $NaHCO_3$ aq and water, dried (Na_2SO_4) and concentrated *in vacuo* to give 2.048 g of crude **20** as a yellow syrup, ν_{max} 3070 (w), 2950 (s), 2880 (s), 1740 (s), 1585 (w), 1460 (s), 1450 (s), 1380 (s), 1370 (s), 1305 (s), 1230 (s), 1190 (s), 1170 (s), 1145 (s), 1090 (s), 1060 (s), 1045 (s), 1020 (s), 1010 (m), 1000 (m), 995 (m), 960 (m), 950 (m), 920 (m), 890 (m), 865 (w), 840 (w), 745 (m), 720 (m), 690 (m) cm^{-1}. This was employed for the next step without further purification.

6, 6-*Ethylenedioxy*-2α, 3α-*isopropylidenedioxy*-24S-*methyl*-5α-*cholest*-22E-*ene* **21a**. A soln of **20** (2.048 g) in MeOH (10 ml) and EtOAc (5 ml) was added to stirred Na–Hg (from 414 mg of Na and 8.2 g of Hg) at −40 ~ −20°. The mixture was stirred for 8 hr at −40 ~ −20° and left to stand for 38 hr at −20°. Then it was poured into water and extracted with ether. The ether soln was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* to give 1.47 g of a gum. This was chromatographed over SiO_2 (Merck Kieselgel, Art 7734, 88 g). Elution with *n*-hexane-EtOAc (96 : 4 ~ 94 : 6) yielded 619 mg of **21a**, ν_{max} 2950 (s), 2880 (s), 1460 (s), 1380 (s), 1370 (s), 1350 (w), 1340 (m), 1305 (m), 1290 (m), 1250 (w), 1230 (s), 1220 (m), 1195 (s), 1190 (s), 1170 (s), 1150 (s), 1125 (s), 1085 (s), 1060 (s), 1050 (s), 1030 (s), 1000 (m), 980 (s), 970 (s), 960 (m), 930 (w), 920 (w), 890 (w), 875 (w), 865 (w), 840 (w), 800 (w), 785 (w), 740 (w), 710 (w) cm^{-1}: δ ($CDCl_3$) 0.67 (3H, s), 0.7–2.6 (m), 3.5–4.4 (6H, m), 5.03–5.35 (2H, m).

2α, 3α-*Dihydroxy*-24S-*methyl*-5α-*cholest*-22E-*en*-6-*one* **21b**. 35% $HClO_4$ soln (30 ml) was added to a soln of **21a** (619 mg) in THF (30 ml) and the mixture was stirred for 1 hr at 50–60°. Then it was poured into water and the precipitated crystals were collected on a filter to give 350 mg of **21b**. The filtrate was extracted with EtOAc. The extract was washed with water and $NaHCO_3$ aq, dried ($MgSO_4$) and concentrated *in vacuo* to give 23 mg of **21b**. The combined crude **21b** was recrystallized from 99% EtOH to give 344 mg (31% from **19**) of **21b** as needles, m.p. 222–225°. $[\alpha]_D^{22}$ + 6.92° (c = 0.997, $CHCl_3$); ν_{max} 3350 (s), 1710 (s), 1700 (sh), 1460 (m), 1450 (m), 1380 (m), 1370 (m), 1330 (w), 1265 (w), 1255 (w), 1230 (w), 1200 (w), 1190 (w), 1150 (w), 1110 (m), 1075 (m), 1055 (m), 1040 (s), 1015 (m), 995 (w), 965 (m), 935 (w), 870 (w) cm^{-1}; δ (400.5 MHz, $CDCl_3$) 0.67 (3H, s), 0.76 (3H, s), 0.81 (3H, d, J = 6.8 Hz), 0.83 (3H, d, J = 6.8 Hz), 0.91 (3H, d, J = 6.8 Hz), 1.01 (3H, d, J = 6.8 Hz), 1.05–2.04 (m), 2.19 (1H, s), 2.29 (1H, dd, J = 4.4 and 13.2 Hz), 2.68 (1H, dd, J = 2.9 and 12.7 Hz), 3.77 (1H, ddd, J = 3.3, 5.0 and 11.7 Hz), 4.05 (1H, dd, J = 2.8 and 3.3 Hz), 5.13 (1H, dd, J = 7.3 and 15.1 Hz), 5.19 (1H, dd, J = 7.3 and 15.1 Hz); MS: m/z 430 (M^+), 415 (M^+–Me), 412 (M^+–H_2O), 387, 369, 345, 331, 303, 287. (Found: C, 76.92; H, 10.75. Calc. for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77).

2α, 3α-*Diacetoxy*-24S-*methyl*-5α-*cholest*-22E-*en*-6-*one* **21c**. Ac_2O (10 ml) and DMAP (0.1 g) were added to a soln of **21b**

(930 mg) in C_5H_5N (10 ml). The mixture was stirred for 17 hr. Then it was poured into ice-dil HCl and extracted with ether. The ether soln was washed with dil HCl, water, $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was recrystallized from 99% EtOH to give 1.06 g (95.3%) of **21c**, m.p. 195–196°, $[\alpha]_D^{24.5}$ + 3.09° (c = 0.683, $CHCl_3$); ν_{max} 1750 (s), 1710 (s), 1460 (m), 1380 (m), 1365 (m), 1340 (w), 1285 (w), 1250 (s), 1245 (m), 1215 (m), 1205 (m), 1200 (w), 1150 (w), 1105 (w), 1090 (w), 1080 (w), 1035 (m), 1020 (m), 995 (w), 990 (w), 960 (w), 935 (w), 900 (w), 880 (w) cm^{-1}; δ ($CDCl_3$) 0.66 (3H, s), 0.7–2.7 (m), 1.95 (3H, s), 2.03 (3H, s), 4.65–5.40 (4H, m); MS: m/z 514 (M^+), 499 (M^+–Me), 471 (M^+–Ac), 454 (M^+–AcOH), 416, 401, 387, 351. (Found: C, 74.57; H, 9.84. Calc. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79%).

2α, 3α-*Diacetoxy*-24S-*Methyl*-22, 23-*epoxy*-5α-*cholestan*-6-*one* **22**. MCPBA (85%, 1.2 g) was added to a soln of **21c** (1.219 g) in CH_2Cl_2 (50 ml) with stirring and ice-cooling. After 30 min the ice-bath was removed and the mixture was stirred for 5 hr at toom temp. The mixture was washed with N-NaOH (100 ml) and water, dried (Na_2SO_4) and concentrated *in vacuo* to give 1.222 g of crystalline **22**. This was recrystallized from CH_2Cl_2-99% EtOH to give 910 mg (72.4%) of **22** as needles, m.p. 203–204.5°, $[\alpha]_D^{24.5}$ + 1.61° (c = 1.077, $CHCl_3$); ν_{max} 1742 (s), 1738 (s), 1703 (s), 1460 (s), 1375 (s), 1350 (w), 1300 (w), 1270 (m), 1255 (s), 1240 (s), 1210 (w), 1200 (w), 1170 (w), 1150 (w), 1120 (w), 1110 (w), 1095 (w), 1040 (s), 1025 (m), 1000 (w), 995 (w), 985 (w), 960 (w), 940 (w), 900 (m), 890 (w), 865 (w), 770 (w), 720 (w) cm^{-1}; δ ($CDCl_3$) 0.66 (3H, s), 0.75–2.85 (m), 1.95 (3H, s), 2.04 (3H, s), 4.65–5.45 (2H, m); MS: m/z 530 (M^+), 470 (M^+–AcOH), 459 (M^+–C_5H_{11}), 428, 415, 395. (Found: C, 72.13; H, 9.44. Calc. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50%).

2α, 3α, 22R, 23R-*Tetraacetoxy*-24S-*methyl*-5α-*cholestan*-6-*one* **23**. A soln of **22** (839 mg) in 30% HBr-AcOH (4 ml) was stirred for 3 hr at room temp. Then it was diluted with water (40 ml), neutralized carefully with solid $NaHCO_3$ and extracted with ether. The ether extract was dried ($MgSO_4$) and concentrated *in vacuo* to give 1.1 g of gummy residue. This was dissolved in AcOH (40 ml)–H_2O (10 ml) and the soln was stirred and heated under reflux at 100–120° for 19 hr. Then it was poured into ice-water, neutralized with solid $NaHCO_3$ and extracted with EtOAc. The EtOAc soln was washed with $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give a gummy residue (1.1 g). This was dissolved in C_5H_5N (5 ml). To this soln were added Ac_2O (5 ml) and DMAP (0.2 g) and the mixture was left to stand at room temp for 21 hr. Then it was poured into ice-dil HCl and extracted with EtOAc. The EtOAc soln was washed with $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 1.0 g of an oil. This was chromatographed over SiO_2 (Merck Kieselgel Art 7734, 75 g, 14.5 × 4 cm). *n*-Hexane–EtOAc (85:15 ~ 2:1) mixture was used for the elution and 200 ml-fractions were collected. Fractions No. 18 ~ 24 (eluted with *n*-hexane–EtOAc = 4 : 1) crystallized. The crude product was recrystallized from EtOAc-*n*-hexane to give 255 mg (25.5% from **22**) of **23**, m.p. 221–222°. This was recrystallized from EtOAc-*n*-hexane to give an analytical sample as rhombs, m.p. 221–224°, $[\alpha]_D^{24.5}$ + 6.81° (c = 0.522, $CHCl_3$); ν_{max} 1735 (s), 1707 (s), 1460 (s), 1370 (s), 1255 (s), 1230 (s), 1225 (s), 1170 (w), 1150 (w), 1100 (w), 1070 (w), 1035 (m), 1020 (m), 975 (w), 950 (w), 935 (w), 900 (w) cm^{-1}; δ (100 MHz, $CDCl_3$) 0.71 (3H, s), 0.75–2.75 (m), 1.93 (6H, s), 1.95 (3H, s), 2.02 (3H, s), 4.83–5.54 (4H, m); MS: m/z 632 (M^+), 572 (M^+–AcOH), 530, 512 (M^+–2AcOH), 489, 429, 388, 387, 345, 327. (Found: C, 67.84; H, 8.92. Calc. for $C_{36}H_{56}O_9$: C, 68.32; H, 8.92%). From fractions No. 13–16, (22*S*, 23*S*)-isomer of **23** was obtained (166 mg, 16.6%), δ ($CDCl_3$) 0.65 (3H, s), 0.75–2.75 (m), 1.95 (3H, s), 2.04 (6H, s), 2.06 (3H, s), 4.60–5.40 (4H, m).

2α, 3α, 22R, 23R-*Tetraacetoxy*-24S-*methyl*-*B*-*homo*-7-*oxa*-5α-*cholestan*-6-*one* (*brassinolide tetraacetate*) **1b**. A soln of CF_3CO_3H was prepared by the addition of $(CF_3CO)_2O$ (1354 μl) to an ice-cooled suspension of 90% H_2O_2 (208 μl) in CH_2Cl_2 (2.1 ml). The peracid soln was added to a soln of **23** (240 mg) in CH_2Cl_2 (12 ml) containing powdered Na_2HPO_4 (1.097 g) with stirring. The exothermic reaction subsided after 30 min. Then the mixture was stirred and heated under reflux for 1 hr. After cooling, ice-water was added to the mixture and CH_2Cl_2 layer was separated. The aq layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 soln was washed with $NaHCO_3$ soln, dried ($MgSO_4$) and concentrated *in vacuo* to give a gum (247 mg). This was chromatographed over SiO_2 (Merck Kieselgel Art 7734, 15 g). Elution with *n*-hexane–EtOAc (4 : 1 ~ 7 : 3) yielded 204 mg (82.9%) of **1b** after recrystallization from 99% EtOH, m.p. 218–220°; $[\alpha]_D^{24}$ + 38.9° (c = 0.711, $CHCl_3$); ν_{max} 1750 (sh), 1740 (s), 1722 (s), 1460 (m), 1440 (m), 1370 (m), 1365 (m), 1325 (w), 1310 (w), 1275 (sh), 1245 (s), 1225 (s), 1180 (m), 1170 (m), 1160 (w), 1135 (m), 1115 (w), 1090 (w), 1070 (m), 1050 (m), 1020 (m), 980 (w), 940 (w), 925 (w), 905 (sh), 890 (w), 760 (w), 690 (w) cm^{-1}; δ (400.5 MHz, $CDCl_3$) δ 0.74 (3H, s), 0.91 (3H, d, J = 6.6 Hz), 0.94 (3H, d, J = 6.4 Hz), 0.96 (3H, d, J = 6.4 Hz), 0.99 (3H, s), 1.01 (3H, d, J = 6.8 Hz), 1.19–1.94 (m), 1.996 (3H, s), 2.001 (3H, s), 2.014 (3H, s), 2.110 (3H, s), 2.29 (1H, ddd, J = 2.2, 12.4 and 15.8 Hz), 3.00 (1H, dd, J = 4.5 and 12.0 Hz), 4.05 (1H, dd, J = 9.4 and 12.5 Hz), 4.13 (1H, dd, J = 1.2 and 12.5 Hz), 4.88 (1H, ddd, J = 2.5, 4.4 and 12.5 Hz), 5.15 (1H, dd, J = 0.4 and 9.3 Hz), 5.33 (1H, dd, J = 1.7 and 8.8 Hz), 5.37 (1H, m); MS: m/z 648 (M^+), 633 (M^+–Me), 588 (M^+–AcOH), 578, 548, 528, 506, 463, 404, 361, 343.

2α, 3α, 22R, 23R-*Tetrahydroxy*-24S-*methyl*-*B*-*homo*-7-*oxa*-5α-*cholestan*-6-*one* (*brassinolide*) **1a**. NaOH soln (381 mg in 1 ml H_2O) was added to a soln of **1b** (184 mg) in MeOH (15 ml). The mixture was stirred and heated under reflux for 3 hr. After cooling. THF (15 ml) was added to the mixture. It was then acidified with 6N-HCl (5 ml). The soln was stirred for 3 hr at room temp. The solvent was removed *in vacuo*. The residue was neutralized with solid $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ soln was dried (Na_2SO_4) and concentrated *in vacuo* to give 116 mg of crystals. This was recrystallized from MeOH to give 78 mg (57.0%) of **1a** as rhombs, m.p. 273–275°, $[\alpha]_D^{24}$ + 41.9° (c = 0.340, $CHCl_3$–MeOH = 9 : 1); ν_{max} 3450 (s), 1725 (m), 1693 (s), 1640 (m), 1460 (s), 1405 (s), 1380 (s), 1330 (s), 1315 (m), 1295 (w), 1278 (m), 1253 (m), 1222 (m), 1183 (s), 1162 (m), 1142 (m), 1122 (m), 1112 (m), 1090 (m), 1062 (s), 1030 (s), 1022 (s), 980 (s), 965 (m), 930 (w), 919 (w) cm^{-1}; ^{1}H-NMR δ (400.5 MHz, C_5D_5N) 0.72 (3H, s), 1.04 (3H, d, J = 6.8 Hz), 1.05 (3H, s), 1.11 (3H, d, J = 6.4 Hz), 1.14 (3H, d, J = 6.8 Hz), 1.21 (3H, d, J = 6.3 Hz), 1.25–2.18 (m), 2.31 (1H, dt, J = 4.0 and 14.5 Hz), 2.52 (1H, ddd, J = 2.0, 12.0 and 14.0 Hz), 3.60 (1H, dd, J = 4.2 and 12.0 Hz), 3.95 (1H, d, J = 8.0 Hz), 3.99–4.11 (3H, m), 4.13 (1H, dd, J = 0.5 and 8.0 Hz), 4.43 (1H, br, s); ^{13}C-NMR δ (25.0 MHz, CD_2Cl_2–CD_3OD = 9 : 1) δ 10.4, 12.0, 12.2, 15.7, 20.9, 21.1, 22.8, 25.2, 28.1, 31.2, 31.9, 37.6, 38.7, 39.8, 40.3, 40.8, 41.6, 43.0, ---, 68.4, 68.5, 71.3, 73.7, 74.7, 178.1. (Found: C, 67.68; H, 9.90. Calc. for $C_{28}H_{48}O_6$: C, 67.43; H, 10.11%).

X-ray analysis of **8b**. A colorless plate-shaped crystal of **8b** was grown from benzene-*n*-hexane solution. Crystal symmetry, space group and preliminary cell dimensions were determined from oscillation and Weissenberg photographs. Accurate lattice constants and diffracted intensities were measured on a Enraf Nonius CAD4 automatic diffractometer with graphite-monochromatized CuKα radiation. Refined cell parameters were calculated by a least squares fit of 24 well-centered reflections. The crystal data are: $C_{35}O_5H_{58}$, M = 558.8, monoclinic, space group $P2_1$, a = 18.228 (2), b = 7.598 (2), c = 12.577 (2) A°, β = 105.2 (1)°, V = 1680.7A°^{3}, μ(CuKα) = 5.69 cm^{-1}. A precise density measurement was not made because of the limited amount of specimen but rough estimation by a floatation method with KI aq as immerse liquid gave ρ_0 = 1.0 ~ 1.2 g/cm^3, from which a plausible density of 1.105 g/cm^3 was calculated assuming two molecules per unit cell.

Three dimensional intensity data for structure determination were collected by the w/θ scanning technique with a scan speed of 4°/min. The crystal with dimensions 0.4 × 0.4 × 0.05 mm was used. Of the 3740 independent reflections within the range up to 2θ = 150°, the intensities of 1891 were judged observed and Lorentz and polarization corrections were applied. No absorption correction was made.

The absolute scale factor and the overall temperature factor were derived by the Wilson method and 380 reflections with |E| > 1.51 were used for phase determination.

The structure was solved by MULTAN.[23] An E map computed

with the phases of the set having the highest combined figures of merit gave no predominant peak, implying the correct structure of the molecule. However, the try to derive a reasonable model from this E map was not successful and so successive structure factors and Fourier syntheses were calculated, starting from 30 highest peaks in the E map. After three cycles, the complete molecule except hydrogens were recognized. The structure was refined by the full matrix least squares method, first with isotropic temperature factors and then with anisotropic ones. Unit weight was given to all reflections. A difference Fourier synthesis revealed 29 H atoms, which were included in the following calculation. Most of non-appeared H atoms were those of Me groups. Three cycles of least squares refinement with anisotropic temp factors for non-H atoms and isotropic ones for H atoms gave the final value of R = 5.8%.

All the calculations for structure determination were carried out on the PDP 11 computer using the SDP (Structure Determination Package) program system compiled by Enraf Nonius Co. The ORTEP computer drawing was calculated at Centry Research Center.

Supplementary material available. Crystallographic data including positional and thermal parameters as well as bond distance and angle calculation have been deposited with the Cambridge Crystallographic Data Centre (CCDC) in England.

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