Synthesis of secasterone and further epimeric 2,3- epoxybrassinosteroids

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As part of a programme directed towards the synthesis of new native brassinosteroids and biologically active analogues we synthesized (22*R*,23*R*,24*S*)-2α,3α-epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one and (22*R*,23*R*,24*S*)-2β,3β-epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one (secasterone) as well as both the corresponding 2,3-epoxides of the (24*R*)-series. In addition, the isomeric 3-dehydro-24-*epi*-teasterone has been prepared. The bioactivity of the new compounds is discussed.

The brassinosteroids represent a new class of steroidal phytohormones with high growth promoting and anti-stress activity. Since the discovery of brassinolide in rape pollen more than 30 other brassinosteroids have been isolated and identified from a broad variety of plants.[1,2] Recently, we found in seeds of *Secale cereale* (rye) the new brassinosteroid secasterone **16** [(22*R*,23*R*,24*S*)-2β,3β-epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one][3] representing the first naturally occurring brassinosteroid with a 2,3-epoxy function. Also the first 3,6-diketo brassinosteroid (3-dehydroteasterone), a possible intermediate in the biosynthetic pathway of brassinosteroids between teasterone and typhasterol,[4] was isolated from lily anthers and leaves of *Distylium racemosum*[5] as well as from grains of *Triticum aestivum* L. (wheat).[6] The GC-MS analysis of such endogenous brassinosteroids, present only in minute amounts in plant material, requires the availability of corresponding reference standards. In this paper we describe the synthesis of the four epimeric brassinosteroids with a 2,3-epoxy function related to castasterone and 24-*epi*-castasterone, as well as the preparation of the isomeric 3-dehydro-24-*epi*-teasterone.

Results and discussion

For the synthesis of both (24*R*)-configurations of 2,3-epoxides **9** and **15** the 3α,5-cyclo Δ^{22}-6-ketone **1** was used as intermediate available from ergosterol *via* mesylation, solvolysis to isoergosterol followed by allylic oxidation to the Δ^7-6-ketone and subsequent Birch reduction.[7] The enantioselective modification of the osmium-catalysed dihydroxylation of (22*E*)-olefin **1** using potassium hexacyanoferrate(III) as the cooxidant and dihydroquinidine *p*-chlorobenzoate (DHQN) as the chiral ligand gave 73% of the desired diol **2** with the (22*R*,23*R*)-configuration[8] essential for the high bioactivity of brassinosteroids. Whereas direct isomerization of the unprotected diol **2** with pyridinium hydrochloride and lithium bromide in dimethylacetamide led to a ring A saturated 3-chloro derivative, the same reaction starting from the isopropylidenedioxy derivative **3** smoothly afforded the desired Δ^2-6-keto acetonide **4**, which was deprotected with 2 mol dm^{-3} HCl to give the 22,23-diol **5**. Epoxidation of **5** with *m*-chloroperbenzoic acid (MCPBA) afforded, *via* attack from the less hindered α-side, stereoselectively (22*R*,23*R*,24*R*)-2α,3α-epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one **9**.

For the synthesis of the (24*S*)-configuration of 2α,3α-epoxy compound **10** the known[9] diacetyl derivative **6** was used. Hydrolysis to the (22*R*,23*R*)-diol **7** followed by epoxidation with MCPBA gave **10**.

To prepare the (24*R*)-2β,3β-epoxide **15** the Δ^2-6-keto acetonide **4** was transformed with *N*-bromosuccinimide (NBS) in dimethoxyethane into the bromohydrin **11**. Acidic deprotection to **12** followed by hydrogen bromide elimination with sodium methoxide led to the desired **15**.

In a similar manner as described for the preparation of 2β,3β-epoxy compound **15** the corresponding (24*S*)-configuration of Δ^2-6-keto acetonide **8**[10] was transformed, *via* the bromohydrin **13**, deprotection to **14** and HBr elimination, to give the native brassinosteroid[3] secasterone **16**.

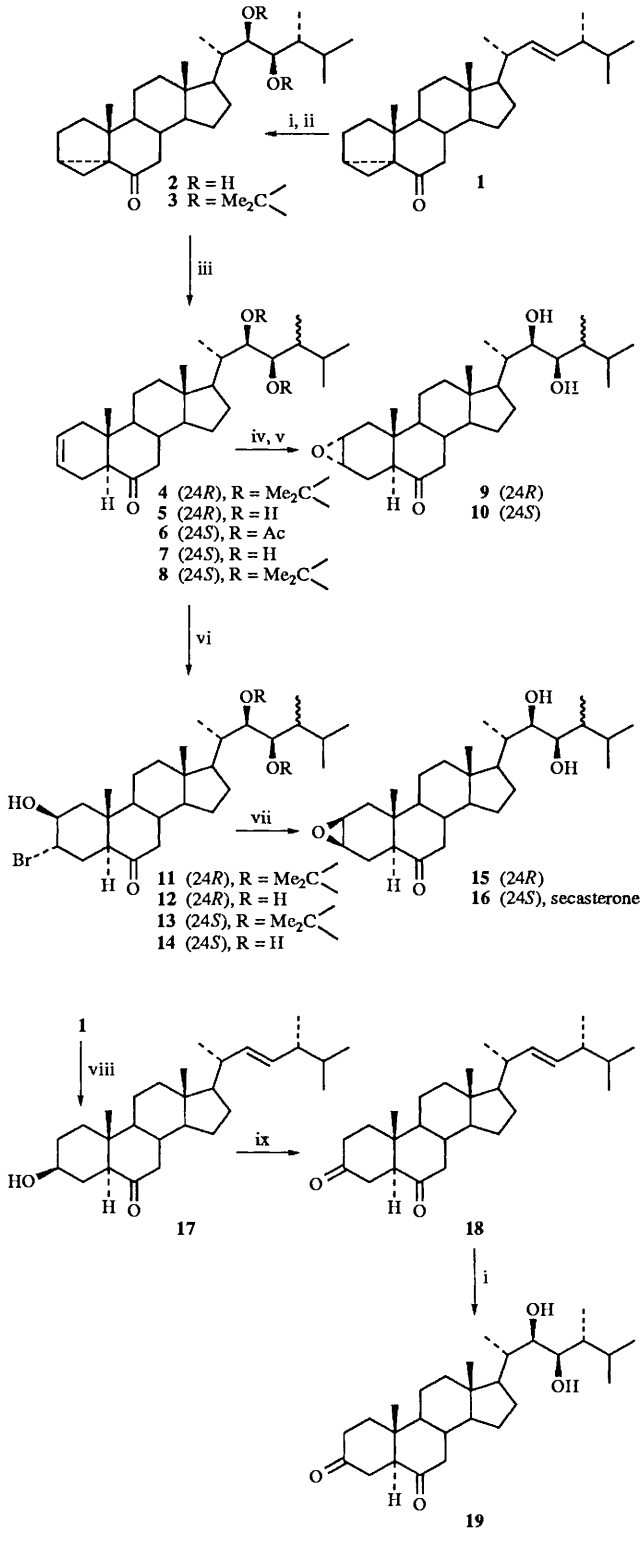
The spectral data of all new compounds are in agreement with the given structures (see Experimental section). In particular, the observed low field shifts (Δppm +0.09) of the 19-methyl singlet in comparison with that of **9** confirms the β-arrangement of the 2,3-epoxy function in compound **15**. The same shift was found for both (24*S*)-epimers **10** and **16**, respectively.

For the synthesis of 3-dehydro-24-*epi*-teasterone **19** 3,5-cyclo ketone **1** was directly dissolved in aqueous H_2SO_4 to give the 3β-hydroxy 6-ketone **17**.[11] Subsequent Jones oxidation led to the 3-dehydro derivative **18**, which afforded upon asymmetric dihydroxylation the 3,6-diketo diol **19**.

To study the phytohormone activity of the new ring A modified 24-*epi*-castasterone analogues the rice lamina inclination bioassay according to the method of Arima *et al.*[12] was used. The obtained results showed that the 2α,3α-epoxy compound **9** at a concentration of 0.01 ppm has a higher activity (88%) than its 2β,3β-epimer **15** (59%) related to 24-*epi*-castasterone as standard (100%). Also the isomeric 3,6-diketo compound **19** exhibits a higher activity (74%) than 2β,3β-epoxide **15**. The question remains open as to whether these activities are due to an *in vivo* biotransformation in the plant material leading to active ring A hydroxylated brassinosteroids[13] such as 24-*epi*-typhasterol.

Experimental

Mps were determined on a Boetius hot stage microscope and are corrected. IR spectra were recorded on a Bruker IFS 28 instrument in Nujol or KBr disks. Optical rotations were measured on a Zeiss-polarimeter Polamat A and are given in units of 10^{-1} deg cm^2 g^{-1}. UV Spectra were measured on an Uvikon 941 Kontron instrument. CD spectra were recorded with a Jasco J 710 spectrometer. Mass spectra (EI-MS, 70 eV) were run on an AMD 402 spectrometer. ^1H NMR spectra were recorded on a Varian UNITY 500 spectrometer at 499.84 MHz in $CDCl_3$ with Me_4Si as an internal standard. *J* Values are given in Hz. Silica gel 60, 0.04–0.063 mm (Merck) was used for flash



Scheme 1 *Reagents:* i, OsO_4, $K_3Fe(CN)_6$, K_2CO_3, DHQD, $MeSO_2NH_2$, Bu'OH–H_2O; ii, $(MeO)_2CMe_2$, *p*TsOH; iii, pyridine–HCl, LiBr, $MeCONMe_2$; iv, HCl, MeOH; v, MCPBA; vi, NBS, DME; vii, MeONa; viii, 5 mol dm^{-3} H_2SO_4, THF; ix, CrO_3, Me_2CO

chromatography. Configurations of (24*S*) compounds were measured as described in ref. 6.

(22*E*,24*R*)-24-Methyl-3α,5-cyclo-5α-cholest-22-en-6-one 1

The title compound was prepared from ergosterol in 40% yield following the procedure described in ref. 7, mp 105–108 °C; $[\alpha]_D^{23}$ +5.1 (*c* 1.82, $CHCl_3$); CD ($CHCl_3$) $\Delta\varepsilon_{292}$ −2.7; λ_{max} (*c* 1.72, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 286 (130).

(22*R*,23*R*,24*R*)-22,23-Dihydroxy-24-methyl-3α,5-cyclo-5α-cholestan-6-one 2

A mixture of olefin **1** (0.400 g, 1 mmol), $K_3Fe(CN)_6$ (2.17 g, 7 mmol, 6 equiv.), anhydrous K_2CO_3 (0.930 g, 7 mmol, 6 equiv.), methanesulfonamide (0.233 g, 2 mmol, 2 equiv.), DHQN (0.117 g, 0.2 mmol, 0.2 equiv.) and OsO_4 (25 mg, 0.1 mmol) in Bu'OH–water, 1:1 (40 cm^3) was stirred at room temp. for 5 days. Solid sodium sulfite (1.0 g) was added, and the mixture was stirred at room temp. for 1 h. Bu'OH was removed under reduced pressure, and the residue was extracted with ethyl acetate (6 × 50 cm^3). The combined organic extracts were washed with H_2SO_4 (0.3 mol dm^{-3}; 3 × 50 cm^3) to recover the ligand and then brine, dried and concentrated. The crude product was purified by flash chromatography on silica gel (80 g). Elution with hexane–ethyl acetate (3:7, v/v) afforded the title compound **2** (0.314 g, 73 %), mp 187–190 °C (lit.,[8] 189–190 °C); $[\alpha]_D^{24}$ +24.1 (*c* 1.02, $CHCl_3$); CD ($CHCl_3$) $\Delta\varepsilon_{300}$ −1.43.

(22*R*,23*R*,24*R*)-22,23-Isopropylidenedioxy-24-methyl-3α,5-cyclo-5α-cholestan-6-one 3

Keto diol **2** (215 mg, 0.5 mmol) in dry ethyl acetate (50 cm^3) was stirred with 2,2-dimethoxypropane (1 cm^3) and toluene-*p*-sulfonic acid (10 mg) for 3 h at room temp. The solvent was removed under reduced pressure, the residue stirred with aqueous K_2CO_3 (5%; 30 cm^3) for 10 min, extracted with ethyl acetate, worked up and than purified by silica gel chromatography. Elution with hexane–ethyl acetate (8:2, v/v) gave the title compound **3** (205 mg, 87%), mp 166–167 °C; $[\alpha]_D^{24}$ +38.1 (*c* 1.40, $CHCl_3$) (Found: C, 78.9; H, 10.5. $C_{31}H_{50}O_3$ requires C, 79.10; H, 10.71%); ν_{max}(film)/cm^{-1} 1680 (CO); λ_{max}(*c* 1.22, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 290 (44); CD ($CHCl_3$) $\Delta\varepsilon_{292}$ −2.88; δ_H 0.71 (3 H, d, *J* 7.0, 28-H_3), 0.72 (3 H, s, 18-H_3), 0.82 (3 H, d, *J* 7.0, 27-H_3), 0.91 (3 H, d, *J* 7.0, 26-H_3), 0.99 (3 H, d, *J* 6.1, 21-H_3), 1.01 (3 H, s, 19-H_3), 1.35 and 1.39 (3 H, s, isopropyl-CH_3), 3.57 (1 H, dd, *J* 9.5, 7.0, 23-H) and 3.95 (1 H, d, *J* 4.7, 22-H); *m/z* (assignment, relative intensity) 471 (M^+ + 1, 8%), 455 (M^+ − 15, 43), 399 (M^+ − 71, 22), 171 (42), 142 (58) and 99 (100) (Found: M^+, 470.3785. $C_{31}H_{50}O_3$. Calc. for *M*, 470.3761).

(22*R*,23*R*,24*R*)-22,23-Isopropylidenedioxy-24-methyl-5α-cholest-2-en-6-one 4

A mixture of 3α,5-cyclo-22,23-acetonide **3** (235 mg, 0.5 mmol), pyridinium hydrochloride (10 mg, 0.1 mmol), anhydrous LiBr (2 mg, 0.025 mmol) and *N*,*N*-dimethylacetamide (3 cm^3) was heated at 160 °C in an argon atmosphere for 2 h. The reaction mixture was poured onto crushed ice, the precipitate was dissolved in ethyl acetate, and the aqueous layer extracted with ethyl acetate. The combined organic extracts were washed with water, dried, concentrated and then purified by silica gel chromatography. Elution with hexane–ethyl acetate (8:2, v/v) afforded the title compound **4** (195 mg, 83%), mp 192–193 °C; $[\alpha]_D^{24}$ +23.1 (*c* 2.81, $CHCl_3$) (Found: C, 78.9; H, 10.5. $C_{31}H_{50}O_3$ requires C, 79.10; H, 10.71%); ν_{max}(Nujol)/cm^{-1} 1708 (CO); λ_{max}(*c* 1.48, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 290 (34); CD (MeOH) $\Delta\varepsilon_{293}$ −1.94; δ_H 0.67 (3 H, s, 18-H_3), 0.71 (3 H, d, *J* 6.4, 28-H_3), 0.71 (3 H, s, 19-H_3), 0.81 (3 H, d, *J* 6.7, 27-H_3), 0.91 (3 H, d, *J* 7.0, 26-H_3), 0.97 (3 H, d, *J* 6.1, 21-H_3), 1.35 and 1.39 (3 H, s, isopropyl-CH_3), 3.57 (1 H, dd, *J* 7.0, 6.8, 23-H), 3.95 (1 H, d, *J* 7.0, 22-H), 5.57 (1 H, m, 2-H) and 5.68 (1 H, m, 3-H); *m/z* 470 (M^+, 3%), 455 (M^+ − 15, 23), 399 (M^+ − 71, 10), 370 (M^+ − 100, 5), 355 (10), 171 (67), 142 (91) and 99 (100).

(22*R*,23*R*,24*R*)-22,23-Dihydroxy-24-methyl-5α-cholest-2-en-6-one 5

A solution of Δ^2-acetonide **4** (119 mg, 0.25 mmol) in methanol (7 cm^3) was stirred with HCl (2 mol dm^{-3}; 5 cm^3) for 5 h at 50 °C. The solvent was removed and the crude product purified by silica gel chromatography. Elution with hexane–ethyl acetate (7:3, v/v) gave the title compound **5** (93 mg, 85%), mp 136–138 °C; $[\alpha]_D^{28}$ +8.8 (*c* 2.27, MeOH) (Found: C, 77.9; H, 10.6. $C_{28}H_{46}O_3$ requires C, 78.09; H, 10.77%); ν_{max}(Nujol)/cm^{-1} 3350 (OH), 1708 (CO) and 1650 (C=C); λ_{max}(*c* 1.48, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 290 (49); CD (MeOH) $\Delta\varepsilon_{293}$ −2.77; δ_H 0.69 (3 H, s, 18-H_3), 0.72 (3 H, s, 19-H_3), 0.85 (3 H, d, *J* 7.0, 28-H_3), 0.87 (3 H, d, *J* 6.7, 27-H_3), 0.92 (3 H, d, *J* 7.0, 26-H_3), 0.99 (3 H, d, *J* 6.7, 21-H_3), 3.42 (1 H, dd, *J* 5.2, 5.2, 23-H), 3.71 (1 H, m, 22-H) and 5.55 (2 H, m, 2- and 3-H); *m*/*z* 430 (M^+, 22%), 415 (M^+ − 15, 9) and 330 (M^+ − 100, 100).

(22*R*,23*R*,24*S*)-22,23-Dihydroxy-24-methyl-5α-cholest-2-en-6-one 7

The (24*S*)-22,23-diacetoxy derivative **6**[9] (56 mg, 0.11 mmol) was refluxed in methanol containing 5% KOH for 1 h. The reaction mixture was neutralized with 6 mol dm^{-3} HCl and extracted with ethyl acetate. The ethyl acetate extract was dried over Na_2SO_4, filtered, evaporated and then crystallized from acetone–hexane to give the 22,23-diol **7** (51 mg, 94%), mp 164 °C (prisms); R_f (silica gel, $CHCl_3$–MeOH, 95:5) 0.61; δ_H 0.851 (3 H, d, *J* 6.8), 0.917 (3 H, d, *J* 6.4), 0.951 (3 H, d, *J* 6.8), 0.971 (3 H, d, *J* 6.8), 2.36 (1 H dd, *J* 13, 4), 3.56 (1 H, dd, *J* 8.8, 1.4), 3.72 (1 H, dd, *J* 8.8, 1.7), 5.57 (1 H, dm, *J* 10.4) and 5.69 (1 H, dm, *J* 10.4); *m*/*z* [as methyl boronate (DB-5 column)] 454 (M^+, 78%), 439 (100), 436 (13), 426 (32) and 155 (15) (Found: M^+, 430.3438. Calc. for *M*, 430.3429).

(22*R*,23*R*,24*R*)-2α,3α-Epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one 9

A solution of Δ^2-keto diol **5** (108 mg, 0.25 mmol) in dry benzene (8 cm^3) and MCPBA (80 mg) was stirred for 1 h at room temp. After dilution with aqueous Na_2SO_3 (5%; 10 cm^3) to destroy the excess of peracid and extraction with ethyl acetate the obtained crude product was purified by silica gel chromatography. Elution with hexane–ethyl acetate (6:4, v/v) gave the title compound **9** (85 mg, 76%), mp 166–169 °C; R_f (silica gel $CHCl_3$–MeOH, 95:5) 0.39; $[\alpha]_D^{27}$ −13.8 (*c* 1.45, MeOH) (Found: C, 75.2; H, 10.3. $C_{28}H_{46}O_4$ requires C, 75.29; H, 10.38%); ν_{max}(Nujol)/cm^{-1} 3400 (OH), 1699 (CO) and 800 (epoxide); λ_{max}(*c* 1.36, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 290 (270); CD (MeOH) $\Delta\varepsilon_{292}$ −1.79; δ_H 0.67 (3 H, s, 18-H_3), 0.71 (3 H, s, 19-H_3), 0.85 (3 H, d, *J* 7.02, 28-H_3), 0.87 (3 H, d, *J* 6.71, 27-H_3), 0.92 (3 H, d, *J* 7.02, 26-H_3), 0.98 (3 H, d, *J* 6.71, 21-H_3), 3.13 (1 H, dd, *J* 5.7, 4.2, 2-H), 3.28 (1 H, t, *J* 1.8, 3-H), 3.41 (1 H, m, 23-H) and 3.70 (1 H, m, 22-H); *m*/*z* 446 (M^+, 9%), 431 (M^+ − 15, 2), 375 (M^+ − 71, 8) and 346 (M^+ − 100, 100) (Found: M^-, 446.3401. Calc. for *M*, 446.3396).

(22*R*,23*R*,24*S*)-2α,3α-Epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one 10

Keto diol **7** (28 mg, 0.065 mmol) was treated with MCPBA (12.7 mg, 0.074 mmol) in dichloromethane (4 cm^3). After 15 h, additional peracid (4 mg) was added to the mixture which was then allowed to stand for 2.5 h. The reaction mixture was diluted with chloroform, washed twice with 1 mol dm^{-3} aq. NaOH, evaporated and crystallized from ethyl acetate–hexane to give the 2α,3α-epoxy derivative **10** (19 mg, 66%), mp 223 °C (prisms); R_f 0.54; δ_H 0.676 (3 H, s), 0.714 (3 H, s), 0.846 (3 H, d, *J* 6.8), 0.909 (3 H, d, *J* 6.4), 0.948 (3 H, d, *J* 6.3), 0.969 (3 H, d, *J* 6.8), 3.12 (1 H, dd, *J* 5.6, 4.2), 3.27 (1 H, dm, *J* 3.4), 3.55 (1 H, ddd, *J* 10.4, 5.2, 1.7; after addition of D_2O, dd, *J* 11.4, 1.7), 3.72 (1 H, ddd, *J* 10.4, 5.5, 2.4; after addition of D_2O, dd, *J* 11.4, 2.4); *m*/*z* [as methyl boronate (DB-5 column)] 470 (M^+, 81%), 454 (41), 439 (42), 426 (14), 316 (17), 260 (9), 245 (32) and 155 (100) (Found: M^+, 446.3389. Calc. for *M*, 446.3382).

(22*R*,23*R*,24*R*)-3α-Bromo-2β-hydroxy-22,23-isopropylidenedioxy-24-methyl-5α-cholestan-6-one 11

NBS (44 mg, 0.24 mmol) was added to a solution of Δ^2-acetonide **4** (51 mg, 0.11 mmol) in dimethoxyethane–water (6:1, 7 cm^3). The mixture was stirred for 1 h at room temp. The reaction mixture was diluted with diethyl ether and then washed with 5% aqueous $Na_2S_2O_3$ and brine. The ethereal layer was dried with $MgSO_4$ and concentrated under reduced pressure to give the bromohydrin acetonide **11** (50 mg, 86%), mp 184–186 °C; $[\alpha]_D^{28}$ +28.7 (*c* 1.29, MeOH) (Found: C, 65.4; H, 8.9; Br, 13.8. $C_{31}H_{51}BrO_4$ requires C, 65.59; H, 9.06; Br, 14.08%); ν_{max}(Nujol)/cm^{-1} 3433 (OH) and 1706 (CO); λ_{max}(*c* 1.29, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 290 (100); CD (MeOH) $\Delta\varepsilon_{291}$ −1.95; δ_H 0.67 (3 H, s, 18-H_3), 0.71 (3 H, d, *J* 7.02, 28-H_3), 0.81 (3 H, d, *J* 6.7, 27-H_3), 0.91 (3 H, d, *J* 7.02, 26-H_3), 0.97 (3 H, s, 19-H_3), 0.99 (3 H, d, *J* 6.10, 21-H_3), 1.35 and 1.39 (3 H, s, isopropyl-CH_3), 2.83 (1 H, dd, *J* 11.9, 2.8, 5α-H), 3.56 (1 H, dd, *J* 9.6, 7.0, 23-H), 3.95 (1 H, d, *J* 6.7, 22-H), 4.24 (1 H, d, *J* 0.9, 2-H) and 4.38 (1 H, s, 3-H); *m*/*z* 553 (M^+ − 15, 14%), 495 (M^+ − 73, 7), 471 (11), 451 (8), 171 (94) and 142 (100).

(22*R*,23*R*,24*R*)-3α-Bromo-2β,22,23-trihydroxy-24-methyl-5α-cholestan-6-one 12

The bromohydrin acetonide **11** (40 mg, 0.07 mmol) in methanol (10 cm^3) was stirred with HCl (2 mol dm^{-3}; 8 cm^3) for 4 h at 50 °C. After removal of methanol under reduced pressure the residue was diluted with ethyl acetate, the organic layer washed with water, dried with Na_2SO_4 and then concentrated under reduced pressure to give the bromohydrin **12** (30 mg, 81%), mp, 197–199 °C; $[\alpha]_D^{28}$ +21.3 (*c* 1.27, MeOH) (Found: C, 63.5; H, 8.8; Br, 14.9. $C_{28}H_{47}BrO_4$ requires C, 63.74; H, 8.98; Br, 15.15%); ν_{max}(KBr)/cm^{-1} 3397 (OH) and 1694 (CO); λ_{max}(*c* 1.27, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 290 (65); CD (MeOH) $\Delta\varepsilon_{292}$ −2.11; δ_H 0.68 (3 H, s, 18-H_3), 0.84 (3 H, d, *J* 7.02, 28-H_3), 0.86 (3 H, d, *J* 6.71, 27-H_3), 0.92 (3 H, d, *J* 6.71, 26-H_3), 0.97 (3 H, d, *J* 6.71, 21-H_3), 0.97 (3 H, s, 19-H_3), 3.38 (1 H, m, 23-H), 3.67 (1 H, dd, *J* 5.9, 1.4, 22-H), 4.17 (1 H, s, 2-H) and 4.39 (1 H, s, 3-H); *m*/*z* 529/527 (M^+ + 1, 1%), 510/508 (M^+ − 18, 1) and 428/426 (M^+ − 100, 100).

(22*R*,23*R*,24*R*)-2β,3β-Epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one 15

To a solution of bromohydrin **12** (26 mg, 0.05 mmol) in methanol (5 cm^3) was added at room temp. sodium methoxide (2.3 mg, 0.1 mmol) in methanol (1 cm^3). After 10 min, the mixture was diluted with water (5 cm^3) and then the methanol was removed under reduced pressure. The aqueous phase was extracted with ethyl acetate, worked up and crystallized to give the 2β,3β-epoxy diol **15** (19 mg, 87%), mp 176–179 °C; R_f 0.57; $[\alpha]_D^{26}$ +9.30 (*c* 2.15, MeOH) (Found: C, 75.1; H, 10.1. $C_{28}H_{46}O_4$ requires C, 75.29; H, 10.38%); ν_{max}(KBr)/cm^{-1} 3515 (OH) and 1708 (CO); λ_{max}(*c* 1.07, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 288 (95); CD (MeOH) $\Delta\varepsilon_{292}$ −2.64; δ_H 0.66 (3 H, s, 18-H_3), 0.80 (3 H, s, 19-H_3), 0.85 (3 H, d, *J* 7.02, 28-H_3), 0.87 (3 H, d, *J* 6.72, 27-H_3), 0.92 (3 H, d, *J* 6.71, 26-H_3), 0.98 (3 H, d, *J* 6.71, 21-H_3), 3.16 (1 H, m, 2-H), 3.24 (1 H, m, 3-H), 3.41 (1 H, m, 23-H) and 3.70 (1 H, m, 22-H); *m*/*z* 446 (M^+, 5%), 375 (M^+ − 71, 4), 357 (375–18, 3) and 346 (M^+ − 100, 100) (Found: M^+, 446.3385. Calc. for *M*, 446.3396).

(22*R*,23*R*,24*S*)-2β,3β-Epoxy-22,23-dihydroxy-24-methyl-5α-cholestan-6-one 16 (secasterone)

The known (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy derivative **8**[10] (18 mg) in dimethoxyethane (4 cm^3) and water (0.6

cm^3) was treated with NBS (50 mg, freshly recrystallized from hot water, *ca.* 90 °C) at room temp. for 3 h. The reaction mixture was diluted with diethyl ether, washed with 5% aq. $Na_2S_2O_3$ (10 cm^3) and then brine, dried over $MgSO_4$, filtered and then concentrated under reduced pressure below 30 °C to give crude product **13**. This was dissolved in methanol (5 cm^3) and tetrahydrofuran (1 cm^3) and the solution was treated with HCl (1.2 mol dm^{-3}; 1 cm^3) at 50 °C for 5 h. The reaction mixture was diluted with chloroform, washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and then concentrated to give crude product **14**, which was dissolved in methanol (4 cm^3) and tetrahydrofuran (1 cm^3). This solution was treated with 28% sodium methoxide (0.2 cm^3 at room temp. for 30 min. The reaction mixture was diluted with chloroform, washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and then concentrated to give a crude product, which was purified by silica gel chromatography. Elution with benzene–ethyl acetate (2:1, v/v) gave the title compound **16** (secasterone, 8 mg), mp 179–180 °C (ethyl acetate–hexane) R_f (benzene–ethyl acetate, 1:1, v/v) 0.34; δ_H 0.675 (3 H, s), 0.806 (3 H, s), 0.846 (3 H, d, *J* 6.8), 0.912 (3 H, d, *J* 6.4), 0.951 (3 H, d, *J* 7.3), 0.969 (3 H, d, *J* 7.3), 2.32 (1 H, dd, *J* 13.2, 3.9), 3.16 (1 H, m), 3.23 (1 H, dd, *J* 5.9, 3.8), 3.56 (1 H, d, *J* 7.7) and 3.72 (1 H, d, *J* 7.7); *m/z* [as methyl boronate (DB-5 column)] 470 (M^+, 66%), 454 (70), 439 (76), 426 (25), 316 (23), 286 (10), 260 (12), 245 (19) and 155 (100) (Found: M^+, 446.3397. Calc. for *M*, 446.3396).

(22*E*,24*R*)-3β-Hydroxy-24-methyl-5α-cholest-22-en-6-one 17

The title compound was prepared in 60% yield starting from **1** according to the literature procedure,[11] mp, 186–187 °C; $[\alpha]_D^{25}$ −35.4 (*c* 0.363, MeOH); ν_{max}(Nujol)/cm^{-1} 3430 (OH) and 1705 (CO); λ_{max}(*c* 1.13, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 289 (70); CD ($CHCl_3$) $\Delta\varepsilon_{293}$ −2.04; δ_H 0.677 (3 H, s, 18-H_3), 0.757 (3 H, s, 19-H_3), 0.817 (3 H, d, *J* 6.71, 28-H_3), 0.833 (3 H, d, *J* 7.02, 27-H_3), 0.908 (3 H, d, *J* 7.02, 26-H_3), 1.011 (3 H, d, *J* 6.71, 21-H_3), 3.577 (1 H, septet, 3-H), 5.141 (1 H, dd, *J* 15.6, 8.0, 23-H) and 5.211 (1 H, dd, *J* 13.3, 7.6, 22-H); *m/z* 414 (M^+, 100%), 399 (M^+ − 15, 12) and 371 (399–28, 17).

(22*E*,24*R*)-24-Methyl-5α-cholest-22-ene-3,6-dione 18

To a stirred solution of 3β-hydroxy ketone **17** (1.37 g, 3.3 mmol) in acetone (100 cm^3 at 0 °C was added dropwise a solution of CrO_3 (1.34 g) in acetone (10 cm^3) and conc. H_2SO_4 (0.1 cm^3). The reaction mixture was stirred for 30 min at 0 °C. After removal of the acetone the product was extracted with ethyl acetate, the organic layer washed with brine, dried (Na_2SO_4) and then evaporated to give a residue which was purified by silica gel chromatography. Elution with hexane–ethyl acetate (8:2, v/v) afforded the diketone **18** (797 mg, 59%), mp 195–198 °C; $[\alpha]_D^{25}$ −36.8 (*c* 1.52, MeOH) (Found: C, 81.3; H, 10.6. $C_{28}H_{44}O_2$ requires C, 81.50; H, 10.75%); ν_{max}(film)/cm^{-1} 1702 (CO); λ_{max}(*c* 1.14, MeOH/nm) (ε/dm^3 mol^{-1} cm^{-1}) 290 (80); CD ($CHCl_3$) $\Delta\varepsilon_{291}$ −2.95; δ_H 0.70 (3 H, s, 18-H_3), 0.82 (3 H, d, *J* 6.7, 28-H_3), 0.83 (3 H, d, *J* 6.7, 27-H_3), 0.91 (3 H, d, *J* 6.7, 26-H_3), 0.96 (3 H, s, 19-H_3), 1.02 (3 H, d, *J* 6.4, 21-H_3), 5.15 (1 H, dd, *J* 15.3, 7.9, 23-H) and 5.22 (1 H, dd, *J* 15.3, 7.3, 22-H); *m/z* 412 (M^+, 83%), 397 (M^+ − 15, 11), 369 (M^+ − 43, 48) and 314 (M^+ − 98, 100).

(22*R*,23*R*,24*R*)-22,23-Dihydroxy-24-methyl-5α-cholestane-3,6-dione 19 (3-dehydro-24-*epi*-teasterone)

Catalytic asymmetric dihydroxylation of diketo olefin **18** (100 mg, 0.24 mmol) as described for **2** gave upon silica gel chromatography and elution with hexane–ethyl acetate (2:8, v/v) the title compound **19** (56 mg, 52%), mp 191–194 °C; R_f 0.81; $[\alpha]_D^{27}$ −19.1 (*c* 1.62, MeOH) (Found: C, 75.1; H, 10.2. $C_{28}H_{46}O_4$ requires C, 75.29; H, 10.38%); λ_{max}(*c* 1.62, MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) 287 (500); CD (MeOH) $\Delta\varepsilon_{294}$ −3.51; δ_H 0.71 (3 H, s, 18-H_3), 0.85 (3 H, d, *J* 7.0, 28-H_3), 0.88 (3 H, d, *J* 7.0, 27-H_3), 0.93 (3 H, d, *J* 6.7, 26-H_3), 0.96 (3 H, s, 19-H_3), 0.99 (3 H, d, *J* 6.4, 21-H_3), 3.42 (1 H, dd, *J* 5.5, 4.8, 23-H) and 3.70 (1 H, d, *J* 4.8, 22-H); *m/z* 446 (M^+, 1%), 375 (M^+ − 71, 4), 357 (375–18, 3) and 346 (M^+ − 100, 100).

Acknowledgements

We thank Dr U. Himmelreich, Dr J. Schmidt and Dr M. Nakayama for mass and NMR spectra. S. T. and T. Y. thank Dr M. Aburatani for the gift of (22*R*,23*R*,24*S*)-22,23-diacetoxy-24-methyl-5α-cholest-2-en-6-one. Financial support from the Deutsche Forschungsgemeinschaft, Germany, is gratefully acknowledged. This work was also supported by a Grant-in-Aid for Cooperative Research from the Ministry of Education, Science and Cultures of Japan.

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Paper 5/01115I
Received 23rd *February* 1995
Accepted 24th *February* 1995