Studies on Steroidal Plant-growth Regulators. Part 29. Osmium Tetroxide-catalysed Asymmetric Dihydroxylation of the (22*E*,24*R*)- and the (22*E*,24*S*)-24-Alkyl Steroidal Unsaturated Side Chain

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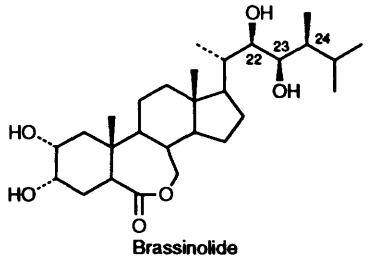
The osmium tetroxide-catalysed asymmetry dihydroxylation of the (22*E*,24*R*)-and (22*E*,24*S*)-24-methyl steroidal unsaturated side chain with dihydroquinidine *p*-chlorobenzoate (DHQD) as chiral ligand gave an 8:1 ratio of (22*R*,23*R*)- and (22*S*,23*S*)-22,23-dihydroxylated products; with a 24-ethyl substituent only a 1.5:1 ratio of (22*R*,23*R*)- and (22*S*, 23*S*)-products was obtained. With the chiral ligand 9-*O*-(9'-phenanthryl) dihydroquinidine (DHQD–PHN), a 8:1 ratio of (22*R*,23*R*)- and (22*S*,23*S*)-products was obtained from the (22*E*,24*S*)-24-ethyl substituted side chain.

Since the structure of brassinolide was determined as (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-24-methyl-7-oxa-β-homo-5-cholestan-6-one,[1] a number of brassinosteroids with and without hydroxy groups or an alkyl substituent in their side-chain have been synthesized.[2]



Brassinolide

The alkyl substituent at C-24, which has a significant influence on the hydroxylation with osmium tetroxide of the C-22 double bond, determines the ratio of (22*R*,23*R*)- and (22*S*,23*S*)-22,23-diols formed. Thus, steroids with a (24*S*)-24-methyl group or without a methyl substituent at C-24 yielded mainly the unnatural (22*S*,23*S*)-isomers, while with a (24*R*)-24-methyl group a 1:1 mixture of isomers was obtained. For the steroid with a (24*S*)-24-ethyl group oxidation yielded mainly the unnatural (22*S*,23*S*)-isomers.[2] Since isomers with the unnatural (22*S*,23*S*)-22,23-dihydroxy groups were inactive or less potent against growth regulator activity,[2] an improved method for obtaining the natural (22*R*,23*R*)-22,23-dihydroxy isomers is required. Recently, Sharpless and his co-workers reported an enantioselective method for the osmium-catalysed asymmetric dihydroxylation of olefins,[3] using potassium ferricyanide as the cooxidant. We were successful in applying this reaction for the first time to the (22*E*)-24-alkyl steroidal unsaturated side-chain, providing the (22*R*,23*R*)-22,23-diols as the major products in three examples (Table 1, entries 1–3, method A).[4] Method A[3] for the dihydroxylation of this unsaturated side chain is not perfect (entry 5, 6), although it is much better than the old one (method C).[2] As is shown in Table 1, an unexpected 8:1 ratio of (22*R*,23*R*) and (22*S*,23*S*) was obtained from the (24*S*)-24-methyl substituted steroidal side chain (entry 3) in contrast to the earlier method (entry 4, method C) in which a 1:4 ratio was obtained. It is noteworthy that a 8:1 ratio of (22*R*,23*R*) and (22*S*,23*S*) was also formed in the (24*R*)-24-methyl substituted steroidal side chain (entries 1 and 2, method A). When the chiral ligand, dihydroquinine *p*-chlorobenzoate (DHQ) replaced dihydroquinidine *p*-chlorobenzoate (DHQD) for the dihydroxylation of the (22*E*, 24*R*)-24-methyl steroids (entry 2, method B), the product ratio for (22*R*,23*R*) and (22*S*,23*S*) was, as expected, reversed. Further, dihydroxylation of the (22*E*,24*S*)-24-ethyl steroid (entry 5, method B), produced the (22*S*,23*S*)-22,23-diols as essentially the sole product. From an inspection of molecular models it is clear that the (24*S*)-24-ethyl and ring D of the steroid nucleus greatly hinder a frontal attack on the side chain by the bulky osmium tetroxide complexed reagent.

Although the ratio of (22*R*,23*R*)- and (22*S*,23*S*)-22,23-diol in the osmium tetroxide-catalysed asymmetric dihydroxylation of the (22*E*,24*S*/24*R*)-24-methyl unsaturated side chain has been greatly improved, the dihydroxylation of the (22*E*,24*S*)-24-ethyl steroid only yielded a 1.5:1 mixture of 22*R*,23*R* and 22*S*,23*S* isomers. Very recently, Sharpless and co-workers reported enhanced product enantiomeric excesses in the osmium tetroxide-catalysed asymmetric dihydroxylation using new chiral ligands.[5] We were also successful in applying this method for the first time to the (22*E*,24*S*)-24-ethyl steroidal side-chain, obtaining the (22*R*,23*R*,24*S*)-22,23-dihydroxy-24-ethyl isomers as major products in two examples (Table 1 entries 5, 6, method D).[6],†

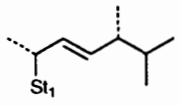
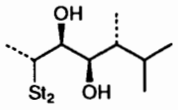
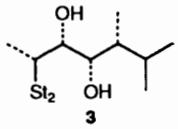
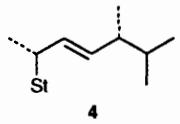
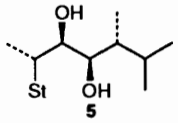
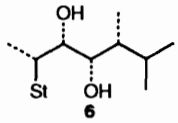
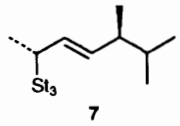
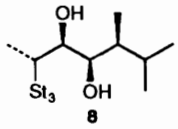
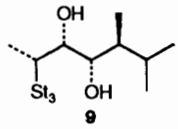
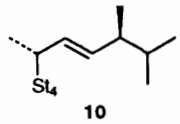
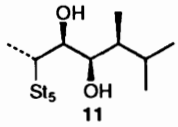
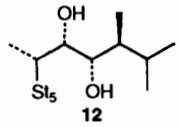
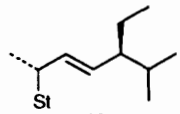
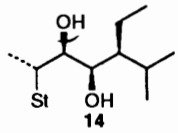
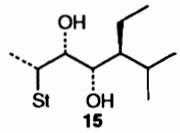
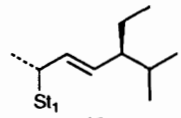
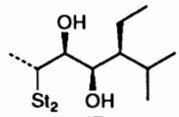
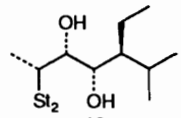
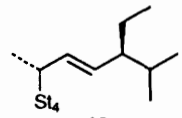
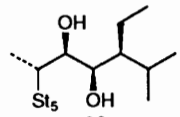
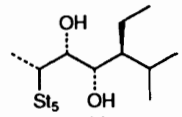
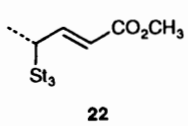
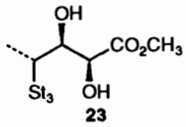
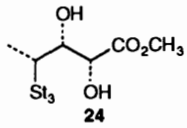
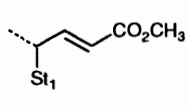
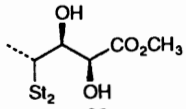
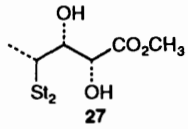
Method D for the dihydroxylation of the (22*E*,24*R*)-24-methyl steroidal side chain is superior (entries 1, 2) to Method A, the catalytic asymmetric dihydroxylation of the steroidal unsaturated side chain being very effective. However, we found that in the examples studied, the rate of reaction and stereoselectivity could be enhanced only by addition of larger amounts of OsO_4 (0.2 mmol) and chiral ligand (0.2–0.4 mmol).

As shown in Table 1, an unexpected 17:1 ratio of (22*R*,23*S*) to (22*S*,23*R*) isomers was obtained from (22*E*)-methyl hyodeoxycholate (entry 8)[6] in comparison with 4:1 for Method A. Similarly, dihydroxylation of (22*E*)-methyl-6-oxo-5α-chola-2,22-dienate **25** with Method D gave the (22*R*,23*S*)-3α,6α,22,23-tetrahydroxy compound **26** as essentially the sole product.[9]

In spite of the presence of a (24*S*)- or (24*R*)-methyl group in the unsaturated side chain, this dihydroxylation method with the new chiral ligand is proving useful for the preparation of the 22*R*,23*R*-bioactive isomers, particularly so for the hydroxylation of the (22*E*,24*S*)-24-ethyl steroidal unsaturated side-chain. New chiral ligands render the (22*E*)-methylhyodeoxy-

† C. Brosa and co-workers reported recently on application of this new chiral ligand (DHQD–PHN) with NMMNO as co-oxidant, to the dihydroxylation of this side chain; they obtained a 2.6:1 ratio of 22*R*,23*R* and 22*S*,23*S* diols.[7]

Table 1 OsO_4-Catalysed asymmetric dihydroxylation of the (22*E*)-steroidal unsaturated side chain[a]

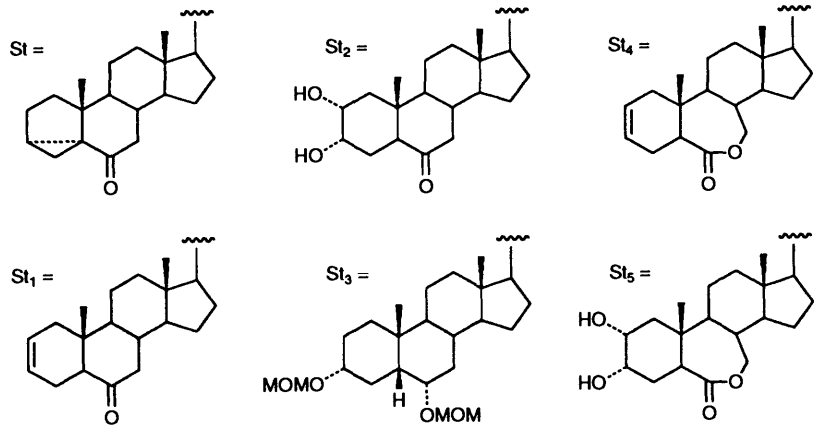
Entry	Olefin	Products	Product ratio[g] (yield)[f]			
			Method A[b]	B[c]	C[d]	D[e]
1	 1	 2  3	8:1 (90%)		3:5[2f] (80%)	13:1 (75%)
2	 4	 5  6	8:1 (94%)	1:9 (84%)		13:1 (78%)
3	 7	 8  9	8:1 (70%)	22*S*:23*S* (82%)		
4	 10	 11  12			1:4[2c]	
5	 13	 14  15	1.5:1 (93%)	22*S*:23*S* (83%)	1:26[2j] (82%)	8:1 (84%)
6	 16	 17  18	1.3:1 (89%)			8:1 (81%)
7	 19	 20  21			1:9[2c] 1:5[2i] (95%)	
8	 22	 23  24	8:1[h,8] (90%)			17:1[h,i] (86%)
9	 25	 26  27				22*R*, 23*S*[9] (76%)[h]

[a] The reaction was carried out at room temperature (RT) in Bu^tOH–H_2O (1:1 v/v), using dihydroquinidine *p*-chlorobenzoate (DHQD) or dihydroquinine *p*-chlorobenzoate (DHQ) or 9-*O*-(9′-phenanthryl)dihydroquinidine (DHQD–PHN, 0.2–0.4 mmol, 0.1–0.2 equiv.), $K_3Fe(CN)_6$ (1.2 mmol, 0.6 equiv.), K_2CO_3 (1.2 mmol, 0.6 equiv.), OsO_4 (0.02 mmol, 0.01 equiv.) and olefin (0.2 mmol, 0.1 equiv.). The reaction mixture was stirred at RT for 4–6 days. [b] Method A: OsO_4–$K_3Fe(CN)_6$–DHQD. [c] Method B: OsO_4–$K_3Fe(CN)_6$–DHQ. [d] Method C: OsO_4–NMMNO. [e] Method D: OsO_4–$K_3Fe(CN)_6$–DHQD–PHN. [f] Isolated yield by flash chromatography. [g] (22*R*,23*R*):(22*S*,23*S*) or (22*R*,23*S*):(22*S*,23*R*). [h] Ligand (0.1 mmol, 0.05 equiv.), $K_3Fe(CN)_6$ (0.6 mmol, 0.3 equiv.), K_2CO_3 (0.6 mmol, 0.3 equiv.), OsO_4 (0.0025 mmol, 0.00125 equiv.), olefin (0.2 mmol, 0.1 equiv.) was used. The reaction time is 24 h. [i] The ratio was determined by ^1H NMR analysis (200 MHz).

cholate a good substrate for this dihydroxylation process, the amounts of chiral ligand and osmium catalyst necessary and the reaction time being diminished greatly (Table 1, entries 8 and 9). The resulting (22*R*,23*S*)-22,23-dihydroxymethylcholate has potential in the synthesis of various brassinosteroids.[8]

Experimental

M.p.s were determined on a Büchi 535 instrument and are uncorrected. IR spectra were run on JMS-01U spectrometer. Optical rotations were measured on Autpol III polarimeter and are recorded in units of 10^{-1} g cm^2. ^1H NMR spectra were



determined with Varian XL-200 spectrometer, using $CDCl_3$ as solvent and TMS as an internal standard; *J* values in Hz. Work-up indicates that the extracts were washed by 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The silica gel H (10–40 μm) was used for flash chromatography. Elemental analyses were performed by the analytical department of this Institute.

(22R,23R,24R)-2α,3α,22,23-*Tetrahydroxy*-24-*methyl*-5α-*ergostan*-6-*one* **2** *and* (22S,23S,24R)-2α,3α-22,23-*Tetrahydroxy*-24-*methyl*-5α-*ergostan*-6-*one* **3**.—*Method A.* To a well-stirred mixture of DHQD (186 mg, 0.4 mmol), potassium ferricyanide (792 mg, 2.4 mmol), potassium carbonate (332 mg, 2.4 mmol) and a *tert*-butyl alcohol solution of osmium tetroxide (0.05 mol dm^{-3}; 0.4 cm^3, 0.02 mmol) in *tert*-butyl alcohol–water (1:1, v/v; 9 cm^3) at room temperature was added all at once (22*E*)-5α-ergost-2,22-dien-6-one **1** (80 mg, 0.2 mmol). The reaction mixture was stirred at room temperature for 4 days after which solid sodium sulfide (600 mg) was added, and the mixture was stirred for 2 h. It was then concentrated to dryness under reduced pressure and the residue was extracted with dichloromethane. Work-up followed by flash column chromatography ($CHCl_3$–MeOH, 20:1), gave compound **2** (73 mg, 80%) and compound **3** (9 mg, 10%): **2**, m.p. 241–242 °C (EtOAc), $[\alpha]_D^{25}$ +0.9 (*c* 0.92, $CHCl_3$) (lit.,[2e] m.p. 241–242 °C, $[\alpha]_D^{23}$ +1); **3**, m.p. 182.5–182.6 °C (EtOAc), $[\alpha]_D^{25}$ −2.5 (*c* 1.2, $CHCl_3$) (lit.,[2e] 184–185 °C), $[\alpha]_D^{23}$ −3). The ^1H NMR, MS and IR data were identical with reported results.[2e]

Method D. The hydroxylation reaction was carried out in the same manner as the previous experiment with DHQD–PHN (803 mg, 1.6 mmol) $K_3Fe(CN)_6$ (3.17 g, 0.96 mmol), K_2CO_3 (1.32 g, 0.96 mmol), the olefin **1** (317 mg, 0.8 mmol), a *tert*-butyl alcohol–water mixture (1:1, v/v; 32 cm^3) and osmium tetroxide (40 mg, 0.16 mmol). The mixture was stirred at room temperature for 6 days, after which work-up gave **2** (261 mg, 70%), m.p. 240–242 °C, and **3** (20 mg, 5%), m.p. 183–184 °C. The spectroscopic data were identical with those described above (*e.g.* DHQD as chiral ligand). Neither showed a m.p. depression when admixed with the product obtained from Method A.

(22R,23R,24R)-22,23-*Dihydroxy*-3α,5-*cycloergostan*-6-*one* **5** *and* (22S,23S,24R)-22,23-*Dihydroxy*-3α,5-*cycloergostan*-6-*one* **6**.—*Method A.* The hydroxylation was carried out in the same way as for compounds **2** and **3**: DHQD (186 mg, 0.4 mmol) $K_3Fe(CN)_6$ (792 mg, 2.4 mmol), K_2CO_3 (332 mg, 2.4 mmol), a *tert*-butyl alcohol solution of osmium tetroxide (0.05 mol dm^{-3}; 0.4 cm^3, 0.02 mmol), a *tert*-butyl alcohol–water mixture (1:1, v/v; 9 cm^3), and (22*E*)-3α,5-cycloergost-22-en-6-one **4** (80 mg, 0.2 mmol) were used. After 4 days at room temperature, the crude product was purified by flash chromatography on silica gel with light petroleum–ethyl acetate (2:1) to give compound **5** (72 mg, 84%) and compound **6** (9 mg, 10%); **5**, m.p. 189.4–190.3 °C, $[\alpha]_D^{25}$ +14.2 (*c* 0.5, $CHCl_3$) (Found: 78.3; H, 11.2. $C_{28}H_{46}O_3$ requires C, 78.09; H, 10.77%); ν_{max}(film)/cm^{-1} 3400 (OH) and 1680 (CO); *m/z* 431 (M^+ + 1), 413 (M^+ − OH) and 395 (M^+ − H_2O − OH); δ_H(200 MHz, $CDCl_3$) 0.73 (3 H, s, 18-H), 0.85 (3 H, d, *J* 7.0, 26-H), 0.87 (3 H, d, *J* 6.7, 28-H), 0.92 (3 H, d, *J* 7.0, 27-H), 0.99 (3 H, *J* 6.8, 21-H), 1.01 (3 H, s, 19-H), 3.42 (1 H, dd, *J* 5, 5.7, 23-H) and 3.71 (1 H, d, *J* 5, 22-H); **6**, m.p. 171.1–171.4 °C; $[\alpha]_D^{25}$ +20.7 (*c* 0.878, $CHCl_3$) (Found: C, 77.7; H, 11.3. $C_{28}H_{46}O_3$ requires C, 78.09; H, 10.77%); ν_{max}(film)/cm^{-1} 3400 (OH) and 1680 (CO); *m/z* 429 (M^+ − 1) and 380 (M^+ − H_2O − OH − Me); δ_H(200 MHz, $CDCl_3$) 0.75 (3 H, s, 18-H), 0.90 (3 H, d, *J* 5.5, 21-H), 1.00 (3 H, s, 19-H), 3.64 (1 H, m, 23-H) and 3.76 (1 H, m, 22-H).

Method B. Olefin **4** (80 mg, 0.2 mmol) was dihydroxylated as described for compounds **5** and **6**, but with DHQ instead of DHQD. After 4 days, work-up provided **5** (7 mg, 8%) and **6** (65 mg, 75%), the spectroscopic data for which were identical with those obtained above.

Method D. The hydroxylation was carried out in the same manner as described for **2** and **3** with DHQD–PHN (210 mg, 0.42 mmol), $K_3Fe(CN)_6$ (400 mg, 1.2 mmol), K_2CO_3 (166 mg, 1.2 mmol), **4** (82 mg, 0.2 mmol), a *tert*-butyl alcohol–water mixture (1:1, v/v; 6 cm^3) and a *tert*-butyl alcohol solution of osmium tetroxide (0.05 mol dm^{-3}; 0.5 cm^3). After the mixture had been stirred at room temperature for 6 days, work-up gave **5** (64 mg, 72%), m.p. 189.7–189.9 °C, and **6** (5 mg, 6%), m.p. 172–172.5 °C. There was no depression of m.p. when these two compounds were mixed with those obtained in the previous experiment. The spectroscopic data were identical with those as described above (*e.g.* DHQD as chiral ligand).

(22R,23R)-22,23-*Dihydroxy*-3α,6α-*bis*(*methoxymethyl*)-5β-*cholestane* **8** *and* (22S,23S)-22,23-*Dihydroxy*-3α,6α-*bis*(*methoxymethyl*)-5β-*cholestane* **9**.—*Method A.* The hydroxylation was carried out in the same way as for **2** and **3** with DHQD (186 mg, 0.4 mmol), $K_3Fe(CN)_6$ (198 mg, 0.6 mmol), K_2CO_3 (83 mg, 0.6 mmol), a *tert*-butyl alcohol solution of osmium tetroxide (0.05 mol dm^{-3}; 0.4 cm^3, 0.02 mmol), a *tert*-butyl alcohol–water mixture (1:1, v/v; 3 cm^3) and (22*E*)-3α,6α-dimethoxy-5β-cholest-22-ene **7** (101 mg, 0.2 mmol). After 3 days at room temperature, the crude product was purified by flash chromatography on silica gel with light petroleum–ethyl acetate (2:1) to give compound **8** (67 mg, 62%) and compound **9** (8 mg, 8%); **8**, ν_{max}(film)/cm^{-1} 3400 (OH); *m/z* 539 (M^+ + 1), 521 (M^+ − OH), 504 (M^+ − 2 OH), 477 (M^+ − MOMO) and 459 (M^+ − MOMO − H_2O); δ_H(200 MHz, $CDCl_3$) 0.70 (3 H,

s, 18-H), 0.90 (3 H, d, *J* 6.9, 21-H), 0.93 (3 H, d, *J* 7.8, 28-H), 0.95 (3 H, s, 19-H), 1.00 (3 H, d, *J* 6.6, 26-H), 1.02 (3 H, d, *J* 6.6, 27-H), 3.36 (3 H, s, MeO), 3.37 (3 H, s, MeO), 3.52 (1 H, m, 3-H), 3.60 (1 H, d, *J* 8.2, 22-H), 3.70 (1 H, d, *J* 8.2, 23-H), 3.95 (1 H, m, 6-H), 4.63 (2 H, s, OCH_2O), 4.67 and 4.71 (2 H, each 1 H, each d, *J* 6.8, OCH_2O); **9**, ν_{max}(film)/cm^{-1} 3400 (OH); *m/z* 521 ($M^+ - OH$), 455 ($M^+ - C_3H_7$), 477 (M^+ – MOMO), 459 (M^+ – MOMO – H_2O), 426 (M^+ – MOMOH – MeOH – H_2O); δ_H(200 MHz, $CDCl_3$) 0.69 (3 H, s, 18-H), 0.90 (3 H, d, *J* 6.7, 26-H), 0.92 (3 H, d, *J* 6.7, 27-H), 0.94 (3 H, d, *J* 5.2, 21-H), 0.96 (3 H, s, 19-H), 1.00 (3 H, d, *J* 8.0, 28-H), 3.38 (3 H, s, MeO), 3.39 (3 H, s, MeO), 3.50–3.74 (3 H, m, 3-H, 22-H, 23-H), 3.94 (1 H, m, 6-H), 4.64 (2 H, s, OCH_2O), 4.68 and 4.76 (2 H, each 1 H, each d, *J* 6.8, OCH_2O).

Method B. (22*E*)-3α,6α-Dimethoxymethyl-5β-cholest-22-ene **7** (101 mg, 0.2 mmol) was dihydroxylated as described for compounds **8** and **9**, with DHQ instead of DHQD. After 3 days, work-up provided **8** (88 mg, 82%), the spectroscopic data for which were identical with those obtained above.

(22R,23R,24S)-22,23-*Dihydroxy*-3α,5-*cyclo*-5α-24-*ethylcholestan*-6-*one* **14** *and* (22S,23S,24S)-22,23-*Dihydroxy*-3α,5-*cyclo*-5α,24-*ethylcholestan*-6-*one* **15**.—*Method A.* (22*E*)-3α,5-Cyclo-5α-24-ethylcholest-22-en-6-one **13** (80 mg, 0.2 mmol) was dihydroxylated as described for compounds **5** and **6**. After 5 days, work-up provided **14** (50 mg, 56%) and **15** (33 mg, 37%); **14**, m.p. 161.1–162.2 °C; $[\alpha]_D^{25}$ +24.89 (*c* 1.675, $CHCl_3$) (Found: C, 78.25; H, 11.3. $C_{29}H_{48}O_3$ requires C, 78.33; H, 10.88%); ν_{max}(film)/cm^{-1} 3400 (OH) and 1680 (CO); *m/z* 445 ($M^+ + 1$), 427 ($M^+ - OH$) and 409 ($M^+ - H_2O - OH$); δ_H(200 MHz, $CDCl_3$) 0.74 (3 H, s, 18-H), 0.93 (3 H, d, *J* 4.5, 21-H), 0.96 (3 H, d, *J* 7.0, 26-H), 0.98 (3 H, d, *J* 7.0, 27-H), 3.61 (1 H, d, *J* 8.0, 23-H), 3.73 (1 H, d, *J* 8.0, 22-H); **15**, m.p. 162.2–163.4 °C, $[\alpha]_D^{25}$ +28.1 (*c* 0.878, $CHCl_3$) (lit.,[2j,k] m.p. 160–160.5 °C, 156–158 °C, $[\alpha]_D^{13}$ +28.5); *m/z* 445 ($M^+ + 1$), 427 ($M^+ - OH$), 409 ($M^+ - H_2O - OH$); δ_H(200 MHz, $CDCl_3$) 0.76 (3 H, s, 18-H), 0.88 (3 H, d, *J* 7.0, 26-H), 0.95 (3 H, d, *J* 7.0, 27-H), 1.02 (3 H, s, 19-H), 1.05 (3 H, d, *J* 7.3, 21-H) and 3.84 (2 H, m, 22-H, 23-H).

Method B. (24*S*)-24-Ethyl-3α,5-cyclo-5α-cholest-22-en-6-one **13** (82 mg, 0.2 mmol) was dihydroxylated as described above for **14** and **15**, with DHQ instead of DHQD. After 4 days, work-up provided **15** (74 mg, 83%), the spectroscopic data for which were identical with those obtained above.

Method D. Compound **13** (82 mg, 0.2 mmol) was dihydroxylated in the same way as described for **5** and **6**, with DHQD–PHN as ligand. After 7 days, work-up provided **14** (66 mg, 74%), m.p. 164–165 °C, $[\alpha]_D^{11}$ +27.8° (*c* 1.06, $CHCl_3$) and **15** (9 mg, 10%), m.p. 161–161.5 °C. The spectroscopic data for compounds **14** and **15** were identical with those obtained above (*e.g.* DHQD as chiral ligand).

(22R,23R,24S)-24-*Ethyl*-2α,3α,22,23-*tetrahydroxy*-5α-*cholestan*-6-*one* **17** *and* (22S,23S,24S)-24-*ethyl*-2α,3α,22,23-*tetrahydroxy*-5α-*cholestan*-6-*one* **18**.—*Method A.* (22*E*,24*S*)-24-Ethyl-5α-cholesta-2,22-dien-6-one **16** (80 mg, 0.2 mmol) was dihydroxylated as described for compounds **2** and **3**. After 5 days, work-up provided **17** (48 mg, 50%) and **18** (37 mg, 39%); **17**, m.p. 247–248.5 °C, $[\alpha]_D^{25}$ +12.5 (*c* 0.82, $CHCl_3$) (lit.,[2c,d] m.p. 243–244 °C, 253–255 °C, $[\alpha]_D^{25}$ +13); **18**, m.p. 208–210 °C, $[\alpha]_D^{25}$ +3.6 (*c* 1.2, $CHCl_3$) (lit.,[2c,d] m.p. 213–214 °C, 200–240 °C, $[\alpha]_D^{25}$ +4). The spectroscopic data were identical with those reported.[2d]

Method D. The hydroxylation was carried out in the same manner as described for compounds **2** and **3**. DHQD–PHN (210 mg, 0.42 mmol), $K_3Fe(CN)_6$ (800 mg, 2.4 mmol), K_2CO_3 (330 mg, 2.4 mmol), **16** (82 mg, 0.2 mmol), a *tert*-butyl alcohol–water mixture (1:1, v/v; 8 cm^3) and a *tert*-butyl alcohol solution of osmium tetroxide (0.05 mol dm^{-3}; 0.8 cm^3, 0.04 mmol) were used. After 6 days at room temperature, work-up gave **17** (68 mg, 71%), m.p. 247–248 °C as needles from EtOAc–MeOH (lit.,[2c,d] 243–244 °C, 253–255 °C) and **18** (9 mg, 9%), m.p. 208–210 °C (lit.,[2c] 213–214 °C). The spectroscopic data for **17** and **18** were identical with those mentioned above (*e.g.* DHQD as chiral ligand).

Methyl (22R,23S)-22,23-*Dihydroxy*-3α,6α-*bis*(*methoxymethyl*)-5β-*cholan*-24-*oate* **23** *and Methyl* (22S,23R)-22,23-*Dihydroxy*-3α,6α-*bis*(*methoxymethyl*)-5β-*cholan*-24-*oate* **24**.—*Method D.* The hydroxylation was carried out in the same manner as described for preparation of compounds **2** and **3** (Method D). DHQD–PHN (50 mg, 0.1 mmol), $K_3Fe(CN)_6$ (198 mg, 0.6 mmol), K_2CO_3 (83 mg, 0.6 mmol), **22** (99 mg, 0.2 mmol), a *tert*-butyl alcohol–water mixture (1:1, v/v; 4 cm^3) and a *tert*-butyl alcohol solution of osmium tetroxide (0.05 mol dm^{-3}; 0.05 cm^3, 0.0025 mmol) were used. After being stirred at room temperature for 24 h, the mixture was worked up to give a 17:1 mixture of **23** and **24** [by ^1H NMR according to the difference of chemical shift at 23-H[8] (compound **23**: δ_H 4.13, **24**: δ_H 4.30)]. This mixture was separated by flash chromatography on silica gel to afford **23** (91 mg, 86%). The spectroscopic data were identical with reported values.[8]

Acknowledgements

The investigation was supported by the National Sciences Foundation of China.

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Paper 3/00399J
Received 21*st January* 1993
Accepted 25*th April* 1993