Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 3913–3918

Studies on the construction of abutasterone-type and 24-*epi*-abutasterone-type side chains employing asymmetric dihydroxylation of (*E*)-20(22),24-cholestadiene

Masayoshi Tsubuki,* Kazuo Iwabuchi and Toshio Honda*

Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

Received 4 October 2005; accepted 1 November 2005

Abstract—The synthesis of abutasterone-type side chain, (20*R*,22*R*,24*S*)-20,22,24,25-tetrahydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholestane **4**, and 24-*epi*-abutasterone-type side chain, (20*R*,22*R*,24*R*)-20,22,24,25-tetrahydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholestane **6**, by means of Sharpless asymmetric dihydroxylation of (*E*)-20(22),24-cholestadiene **1** is described. Construction of abutasterone-type side chain **4** was carried out by selective mono-dihydroxylation of diene **1** with $(DHQ)_2AQN$, followed by dihydroxylation of the corresponding (24*S*)-hydroxy-20(22)-cholestene **2** with $(DHQD)_2AQN$. In contrast, bis-dihydroxylation of **1** with either $(DHQD)_2PHAL$ or $(DHQD)_2AQN$ preferentially occurs to produce 24-*epi*-abutasterone-type side chain **6**.

1. Introduction

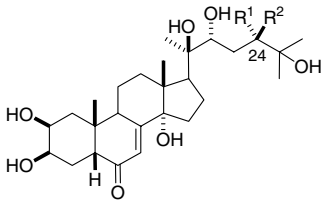
Ecdysteroids are steroid hormones that regulate aspects of development, metamorphosis, reproduction, and diapause in all life cycle stages from newly laid eggs to adult insects.[1] Ecdysteroids are the steroid hormones of all classes of arthropods and possibly of other invertebrates. Ecdysteroids are found not only in insects, crustaceans, and other animal sources but also in many plant species. More than 300 different types of ecdysteroids have so far been isolated from animal and plant sources.

Although ecdysteroids are structurally quite different from mammalian steroids, the pharmacological effects of ecdysteroids on mammalians, including humans, have been widely reported,[2] such as growth-promoting effects, effects on cellular proliferation and differentiation, stimulatory effects of protein synthesis, improvements of both glucose and lipid metabolism, and neuromodulatory actions. Especially, ecdysteroids are widely used as inducers for gene-switch systems, which could be used in human gene therapy. Studies on these pharmacological effects have been considerably supported by the availability of ecdysteroids including their congeners. Since rare ecdysteroids have often been found as minor constituents, the development of a general method for the synthesis of ecdysteroids has become an important factor for quantitative bioassay.[3]

Abutasterone was isolated from the Amazonian plant, *Abuta velutina*,[4] and its epimer, 24-*epi*-abutasterone, was also isolated from *Vitex canescens*.[5] Furthermore, isolation of 24-hydroxylated ecdysteroids,[6] such as nusilsterone[6c] and pterosterone,[6d] have been reported. The commonly hydroxylated sites on the side chains are 20*R*, 22*R*, and 25, making 24-hydroxylated ecdysteroids quite rare. As part of our continuing studies on the synthesis of physiologically active steroids with highly oxygenated side chains,[7] we focused our attention on the synthesis of the abutasterone-type and its 24-epimer-type side chains. Herein, we report the construction of abutasterone-type and 24-*epi*-abutasterone-type side chains by means of Sharpless asymmetric dihydroxylation.[8] The strategy for the synthesis of both (20*R*,22*R*,24*S*)-tetraol **4**, an abutasterone-type side chain, and (20*R*,22*R*,24*R*)-tetraol **6**, a 24-*epi*-abutasterone-type side chain, was envisaged to employ (*E*)-20(22),24-cholestadiene **1**, readily prepared by Wittig olefination of the 20-keto steroid. Mono-dihydroxylation of diene **1** followed by successive dihydroxylation

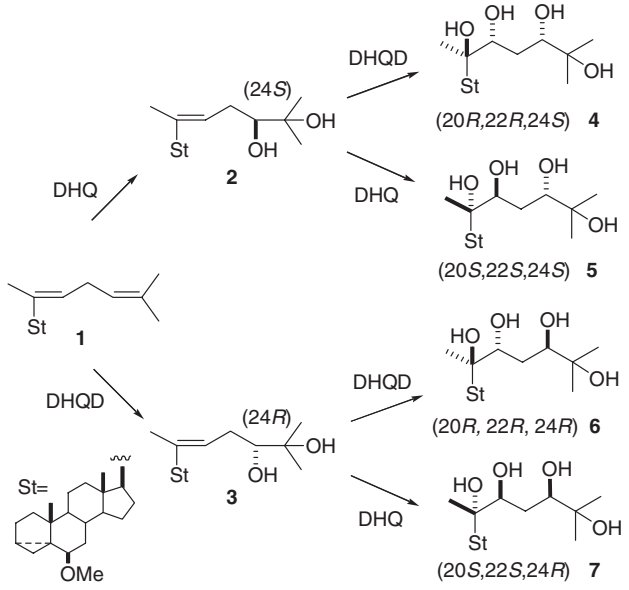
* Corresponding authors. Tel.: +81 3 5498 5793; fax: +81 3 3787 0036 (M.T.); tel.: +81 3 5498 5791; fax: +81 3 3787 0036 (T.H.); e-mail addresses: tsubuki@hoshi.ac.jp; honda@hoshi.ac.jp

0957-4166/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2005.11.001



abutasterone R^1=OH, R^2=H
24-*epi*-abutasterone R^1=H, R^2=OH

of 20(22)-alkenes **2** and **3** with suitable chiral ligand combinations would occur regio- and diastereoselectively to provide **4** and **6** (Scheme 1).[9]

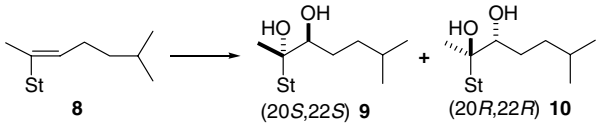


Scheme 1. Synthetic plan for the construction of abutasterone-type and 24-*epi*-abutasterone-type side chains.

2. Results and discussion

Ikekawa[10] and Gut[11] independently reported the dihydroxylation of (*E*)-20(22)-steroidal olefins with osmium tetroxide (OsO_4) leading to (20*S*,22*S*)-diols (unnatural form) preferentially. However extensive studies on the asymmetric dihydroxylation of (*E*)-20(22)-steroidal olefins have not yet been carried out. Thus, we first investigated the asymmetric dihydroxylation of (*E*)-20(22)-cholestene **8**,[11] as shown in Table 1. Since the reaction rate with a catalytic amount of OsO_4 and cooxidant was very sluggish, we used a stoichiometric amount of OsO_4 for the dihydroxylation. As expected, the dihydroxylation of **8** with dihydroquinine (DHQ) derived ligands would be matched reactions to afford exclusively **9** (entries 2, 4, and 8). In contrast, dihydroquinidine (DHQD) derived ligands, $(DHQD)_2PHAL$, DHQD-CLB, and $(DHQD)_2AQN$, gave moderate results due to mismatched reactions (entries 3, 5, and 9). Regarding the preparation of (20*R*,22*R*)-diol **10**[10b] (natural form), $(DHQD)_2AQN$ was the ligand of choice for the mismatched reaction.

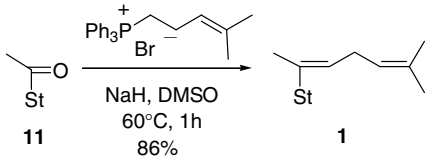
Table 1. Asymmetric dihydroxylation of (*E*)-20(22)-cholestene **8**[a]



Entry	Ligand	Yield (%)	Ratio of products[b]	
			9	**10**
1	None	66	96	4
2	$(DHQ)_2PHAL$	81	100	0
3	$(DHQD)_2PHAL$	90	64	36
4	DHQ-CLB	79	100	0
5	DHQD-CLB	74	59	41
6	$(DHQ)_2PYR$	84	97	3
7	$(DHQD)_2PYR$	84	87	13
8	$(DHQ)_2AQN$	92	100	0
9	$(DHQD)_2AQN$	83	57	43

[a] All reactions were run with a stoichiometric amount of osmium tetroxide.
[b] Determined by 270 MHz ^{1}H NMR spectral analysis.

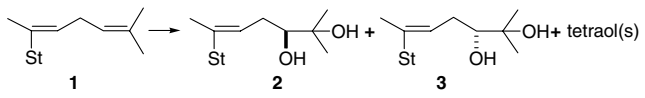
(*E*)-20(22),24-Cholestadiene **1**, a key starting material, was prepared by Wittig olefination of 20-keto steroid **11**[10b] with 4-methyl-3-pentenylidenetriphenylphosphorane[12] in 86% yield (Eq. 1). The Wittig olefination of sterically hindered 20-keto steroids with unstabilized ylides occurs stereoselectively to give the *E*-isomers with no detectable *Z*-isomers.[13] The stereochemistry of the olefin was deduced by the chemical shifts of the 18- and 21-methyl protons, which appear at 0.58 and 1.64 ppm, respectively. The signals for the 18- and 21-methyl protons in the *E*-isomers occur at higher fields than would be expected for the *Z*-isomers (18-methyl protons: 0.65–0.78 ppm; 21-methyl protons: 1.68–1.71 ppm).[13,14]



(1)

Selective mono-dihydroxylation of (*E*)-20(22),24-cholestadiene **1** was examined as shown in Table 2. It is well known that the regioselectivity of the mono-dihydroxylation of a polyene is determined by electronic and steric effects.[8c] We supposed that steric effects might play a decisive role in controlling site-selectivity with respect to electronically similar double bonds and thus the less hindered 24-olefin could be osmylated preferentially. In the absence of a chiral ligand treatment of **1** with a stoichiometric amount of OsO_4 disappointingly gave almost equal amounts of mixture (24*R*)-diol **3** and tetraol **5** (entry 1). The use of DHQ derived ligands led to the formation of bis-dihydroxylated tetraols as minor products (entries 2, 4, 6, and 8). Pleasingly, $(DHQD)_2$-PHAL and $(DHQD)_2AQN$ gave complete regio- and diastereo-selectivities to produce only (24*R*)-hydroxylated product **3** (entries 3 and 9). A good level of regio- and diastereo-selection was observed as (24*S*)-diol **2** was found to be the major product in a ratio of (**2**/**3**/**5** =)82:2:16 with $(DHQ)_2AQN$ as the ligand (entry 8).

Table 2. Mono-dihydroxylation of (*E*)-20(22),24-cholestadiene **1**[a]

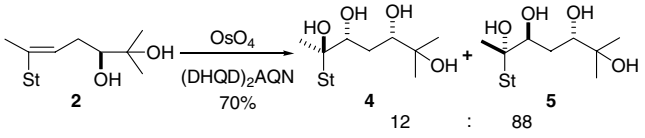


Entry	Ligand	Yield (%)	Ratio of products[b]		
			2	**3**	Tetraol(s)
1	None	57	0	47	53[c]
2	$(DHQ)_2PHAL$	94	53	23	24[c]
3	$(DHQD)_2PHAL$	87	0	100	0
4	DHQ-CLB	48	52	0	48[c]
5	DHQD-CLB	84	0	52	48[d]
6	$(DHQ)_2PYR$	47	0	73	27[e]
7	$(DHQD)_2PYR$	48	0	39	61[f]
8	$(DHQ)_2AQN$	88	82	2	16[c]
9	$(DHQD)_2AQN$	90	0	100	0

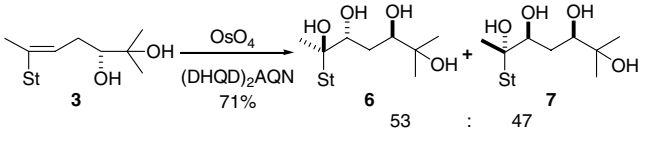
[a] All reactions were run with a stoichiometric amount of osmium tetroxide.
[b] Determined by 270 MHz ^1H NMR spectral analysis.
[c] Tetraol **5** mostly formed.
[d] **4/5/6/7** = 0:25:9:14.
[e] **4/5/6/7** = 0:13.5:0:13.5.
[f] **4/5/6/7** = 49:0:0:12.

Taking account of the formation of (20*S*,22*S*,24*S*)-tetraol **5**, the diastereoselection at the 24-position would be perfect in the reactions employing DHQ-CLB and $(DHQ)_2AQN$ (entries 4 and 8). The high diastereoselectivity observed for the dihydroxylation of **1** with the PHAL and AQN ligands is in accordance with the stereoselectivity reported in the asymmetric dihydroxylation of desmosterol benzoate using the PHAL ligands.[8c]

With both the requisite (24*S*)- and (24*R*)-diols **2** and **3** in hand, we carried out the asymmetric dihydroxylation with $(DHQD)_2AQN$ selected as the ligand of choice (Table 1; entry 9) leading to abutasterone- and 24-*epi*-abutasterone-type side chains **4** and **6** as shown in Eqs. 2 and 3. The reaction of **2** using $(DHQD)_2AQN$ displayed a lower level of mismatched diastereoselectivity (12:88). This result suggests that the nature of hydroxyl group at the 24-position could have a significant influence on the stereochemical course of the reaction as compared to previous results.[15] In contrast, a slight increase in the ratio (53:47) favoring the natural diastereomer **6** was observed when the reaction of **3** was performed with $(DHQD)_2AQN$. Unfortunately, we could not find a combination of **2** and a chiral ligand to override the intrinsic diastereofacial selectivity.



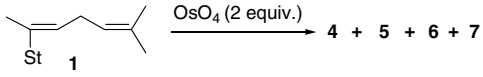
(2)



(3)

For the purpose of producing 24-*epi*-abutasterone-type side chain **6** in a single step, we next investigated bis-dihydroxylation of (*E*)-20(22),24-cholestadiene **1** and the results shown in Table 3. Treatment with 2 equiv of OsO_4 in the absence of a ligand afforded a slightly higher amount of **6** by a single step than the amount estimated by sequential mono-dihydroxylations (entry 1). Moderate levels of diastereoselection were observed when the reactions with $(DHQD)_2PHAL$ and $(DHQD)_2AQN$ were performed (entries 2 and 3). Although the reason for the formation of (20*S*,22*S*,24*S*)-**5** in the presence of the DHQD ligands is unclear, dihydroxylation at the 20-position might compete poorly with preferred dihydroxylation at the 24-position. As far as the preparation of desired **6**, the levels of diastereoselectivities in both the bis- and sequential mono-dihydroxylations using $(DHQD)_2PHAL$ and $(DHQD)_2AQN$ were almost equal in magnitude.

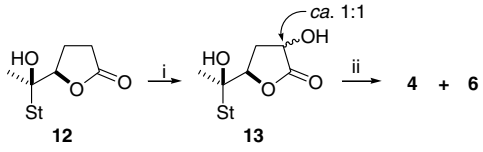
Table 3. Bis-dihydroxylation of (*E*)-20(22),24-cholestadiene **1**



Entry	Ligand	Yield (%)	Ratio of products[a]			
			4	**5**	**6**	**7**
1	None	59	0	59	20	21
2	$(DHQD)_2PHAL$	72	0	10	52	38
3	$(DHQD)_2AQN$	70	0	20	48	32

[a] Determined by 270 MHz ^1H NMR spectral analysis.

We furthermore carried out an alternative synthesis of the abutasterone- and its 24-epimer-type side chains **4** and **6** in order to confirm the structures of tetraols **4**–**7** as follows (Scheme 2). Hydroxylation of the ester enolate, prepared by treatment of (20*R*,22*R*)-γ-lactone **12**[7g] with LDA, using Vedejs' reagent, MoOPH,[16] afforded an inseparable diastereomeric mixture of α-hydroxy lactone **13** in a ratio of ca. 1:1. Addition of MeMgBr to **13** gave tetraols **4** and **6** in 43 and 10% yields, respectively. The spectroscopic data of both **4** and **6** are identical with those obtained in the asymmetric dihydroxylation of (*E*)-20(22),24-cholestadiene **1**.



Scheme 2. Reagents and conditions: (i) LDA, MoOPH, THF, 59%; (ii) MeMgBr, THF, **4** for 43%, **6** for 10%.

3. Conclusion

We have disclosed a new method for the synthesis of abutasterone-type and 24-*epi*-abutasterone-type side chains **4** and **6** employing the Sharpless asymmetric dihydroxyl-

ation of (*E*)-20(22),24-cholestadiene **1**. Although the levels of diastereoselectivities in the dihydroxylation are moderate, this method provides a facile access to versatile 24-hydroxylated ecdysteroids. These 24-hydroxylated steroids could be key intermediates for the synthesis of not only ecdysteroids but also other physiologically active steroids with highly oxygenated side chains, such as cephalostatin. Recent studies toward the synthesis of potent LXR activating steroids have shown the importance of 24-hydroxy steroids as highly versatile intermediates.[17] Further investigation directed toward the synthesis of 24-hydroxylated ecdysteroids is underway in our laboratory.

4. Experimental

4.1. General

IR spectra were obtained using a JASCO FT/IR-200 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL JNM-LA270 (^{1}H NMR: 270 MHz, ^{13}C NMR: 67.8 MHz) or a JEOL JNM-LA500 (^{1}H NMR: 500 MHz, ^{13}C NMR: 125 MHz) instrument for solutions in $CDCl_3$, and chemical shifts are reported on the δ scale using TMS as an internal standard of δ 0.00 for ^{1}H NMR spectra and $CDCl_3$ as an internal standard of δ 77.00 for ^{13}C NMR spectra, respectively. MS spectra were measured with a JEOL JMS-D300 or a JEOL JMS-SX102 spectrometer. Optical rotations were taken with a JASCO DIP-360 polarimeter.

4.1.1. General procedure for the dihydroxylation of the olefins 1–3 and 8. To a stirred solution of olefin (0.05 mmol) and chiral ligand (no use in the case of entry 1 in Tables 1 and 2) (0.055 mmol) in *t*-BuOH (1.0 mL) was added dropwise a solution of OsO_4 (0.02 M in *t*-BuOH, 2.75 mL, 0.055 mmol) at room temperature and the mixture stirred until the starting material was consumed (usually 1–3 h). A solution of sodium bisulfite (52 mg, 0.5 mmol) in water (1.0 mL) and pyridine (1.0 mL) were added to the mixture and the reaction mixture stirred for 0.5 h. The crude product was extracted with AcOEt. The extract was washed successively with brine, aqueous saturated potassium hydrogen sulfate, aqueous saturated sodium hydrogen carbonate, and brine, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane–AcOEt (for **2**, **3**, **9**, and **10**, 75:25, v/v; for **4**–**7**, 50:50, v/v) afforded a mixture of di- or tetra-hydroxylated compounds. The ratio of the tetraols was determined by the analysis of their ^{1}H NMR spectra with the results shown in Tables 1, 2 and Eqs. 2 and 3.

4.1.2. General procedure for the bis-dihydroxylation of the olefin 1 in Table 3. Bis-dihydroxylation of **1** (40 mg, 0.1 mmol) with OsO_4 (0.02 M in *t*-BuOH, 10 mL, 0.2 mmol) and chiral ligand (not used in the case of entry 1) (0.2 mmol) in *t*-BuOH (2.0 mL) was performed as above to afford the crude products. Purification of the crude product by column chromatography on silica gel eluting with hexane–AcOEt (50:50, v/v) afforded a mixture of tetraols **4**–**7**. The ratio of the tetraols was determined by the analysis of their ^{1}H NMR spectra with the results shown in Table 3.

4.1.3. (20*E*)-6β-Methoxy-3α,5-*cyclo*-5α-cholesta-20(22),24-diene 1. To a stirred solution of phosphorane,[12] prepared by treatment of 4-methyl-3-pentenyltriphenylphosphonium bromide (6.8 g, 16 mmol) with sodium hydride (ca. 60% purity, 726 mg, 18 mmol) in DMSO (17 mL), was added a solution of ketone **11**[10b] (660 mg, 2 mmol) in benzene (3 mL) at 60 °C and the mixture stirred at the same temperature for 1 h. After cooling, aqueous saturated ammonium chloride was added to the mixture in water-bath and the mixture extracted with AcOEt. The extract was washed with aqueous saturated ammonium chloride and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane–AcOEt (99:1, v/v) afforded diene **1** (678 mg, 86%) a colorless glass. $[\alpha]_D^{24} = -33.9$ (*c* 1.75, $CHCl_3$); IR: 2930, 1455, 1380, 1100 cm^{-1}; ^{1}H NMR (500 MHz) δ 0.44 (1H, dd, $J = 8.1$, 5.1 Hz, 4α-H), 0.58 (3H, s, 18-H_3), 0.65 (1H, t, $J = 5.1$ Hz, 4β-H), 0.81–0.94 (3H, m), 1.02 (3H, s, 19-H_3), 1.04–1.85 (14H, m), 1.63 and 1.69 (each 3H, each br s, 26-H_3 and 27-H_3), 1.64 (3H, br s, 21-H_3), 1.91 (1H, dt, $J = 13.4$, 2.7 Hz, 7-*H*H), 2.01 (1H, t, $J = 9.8$ Hz, 17-H), 2.72 (2H, t, $J = 7.0$ Hz, 23-H_2), 2.77 (1H, t, $J = 2.7$ Hz, 6-H), 3.30 (3H, s, OCH_3), 5.11 (1H, tt, $J = 7.0$, 1.2 Hz, 24-H), 5.15 (1H, t, $J = 7.0$ Hz, 22-H); ^{13}C NMR (125 MHz) δ 13.1, 13.2, 17.7, 17.9, 19.3, 21.5, 22.7, 24.2, 24.7, 24.9, 25.7, 27.1, 30.7, 33.3, 35.0, 35.3, 39.1, 43.4, 43.9, 48.3, 56.1, 56.5, 59.0, 82.4, 123.6, 124.0, 131.2, 134.5; MS (EI) (rel. int.): 93 (100, base), 396 (5.4, M^+); HRMS (EI) calcd for $C_{28}H_{44}O$: 396.3392; found: 396.3389.

4.1.4. (20*E*,24*S*)-24,25-Dihydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholest-20(22)-ene 2. A colorless glass; $[\alpha]_D^{24} = +12.9$ (*c* 1.00, $CHCl_3$); IR: 3440, 2930, 1455, 1380, 1100 cm^{-1}; ^{1}H NMR (500 MHz) δ 0.44 (1H, dd, $J = 7.9$, 4.8 Hz, 4α-H), 0.61 (3H, s, 18-H_3), 0.66 (1H, t, $J = 4.8$ Hz, 4β-H), 0.82–0.95 (3H, m), 1.03 (3H, s, 19-H_3), 1.04–1.87 (14H, m), 1.20 and 1.25 (each 3H, each s, 26-H_3 and 27-H_3), 1.68 (3H, br s, 21-H_3), 1.91 (1H, dt, $J = 13.4$, 2.7 Hz, 7-*H*H), 2.03 (1H, br s, OH), 2.08 (1H, t, $J = 9.7$ Hz, 17-H), 2.17 (1H, br s, OH), 2.24 (2H, distorted t, $J = 8.5$ Hz, 23-H_2), 2.78 (1H, t, $J = 2.7$ Hz, 6-H), 3.33 (3H, s, OCH_3), 3.42 (1H, dd, $J = 8.9$, 4.3 Hz, 24-H), 5.28 (1H, t, $J = 7.3$ Hz, 22-H); ^{13}C NMR (125 MHz) δ 13.1, 13.5, 18.2, 19.3, 21.4, 22.7, 23.7, 24.1, 24.8, 24.9, 26.6, 30.6, 30.7, 33.3, 35.1, 35.3, 39.2, 43.4, 43.9, 48.3, 56.1, 56.6, 59.1, 72.6, 77.8, 82.4, 121.1, 139.0; MS (CI) (rel. int.): 395 (100, base), 431 (6.6, M+1); HRMS (CI) calcd for $C_{28}H_{46}O_3$+H: 431.3525; found: 431.3514.

4.1.5. (20*E*,24*R*)-24,25-Dihydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholest-20(22)-ene 3. A colorless glass; $[\alpha]_D^{25} = -44.1$ (*c* 0.85, $CHCl_3$); IR: 3420, 2930, 1455, 1380, 1100 cm^{-1}; ^{1}H NMR (500 MHz) δ 0.44 (1H, dd, $J = 7.9$, 4.8 Hz, 4α-H), 0.61 (3H, s, 18-H_3), 0.65 (1H,

t, $J = 4.8$ Hz, 4β-H), 0.82–0.94 (3H, m), 1.03 (3H, s, 19-H_3), 1.00–1.87 (14H, m), 1.20 and 1.25 (each 3H, each s, 26-H_3 and 27-H_3), 1.68 (3H, br s, 21-H_3), 1.91 (1H, dt, $J = 13.4$, 2.7 Hz, 7-*H*H), 2.03 (1H, br s, OH), 2.08 (1H, t, $J = 9.7$ Hz, 17-H), 2.15 (1H, br s, OH), 2.24 (2H, distorted t, $J = 7.3$ Hz, 23-H_2), 2.78 (1H, t, $J = 2.4$ Hz, 6-H), 3.33 (3H, s, OCH_3), 3.42 (1H, dd, $J = 7.3$, 5.5 Hz, 24-H), 5.28 (1H, t, $J = 7.3$ Hz, 22-H); ^{13}C NMR (125 MHz) δ 13.1, 13.4, 18.2, 19.3, 21.4, 22.7, 23.7, 24.1, 24.8, 24.9, 26.6, 30.5, 30.7, 33.3, 35.0, 35.2, 39.2, 43.4, 43.9, 48.2, 56.0, 56.5, 59.2, 72.5, 77.8, 82.3, 121.0, 139.0; MS (EI) (rel. int.): 398 (100, base), 430 (23.3, M^+); HRMS (EI) calcd for $C_{28}H_{46}O_3$: 430.3447; found: 430.3449.

4.1.6. (20*R*,22*R*,24*S*)-20,22,24,25-Tetrahydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholestane 4. A colorless glass; $[\alpha]_D^{21} = +20.6$ (*c* 0.48, $CHCl_3$); IR: 3425, 2930, 1455, 1380, 1100 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.44 (1H, dd, $J = 7.9$, 4.8 Hz, 4α-H), 0.65 (1H, t, $J = 4.8$ Hz, 4β-H), 0.80–0.90 (3H, m), 0.93 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.04–1.82 (20H, m), 1.10 (3H, s, 21-H_3), 1.18 and 1.23 (each 3H, each s, 26 and 27-H_3), 1.89 (1H, dt, $J = 13.4$, 2.7 Hz, 7-*H*H), 2.09 (1H, dt, $J = 12.5$, 3.0 Hz, 12-*H*H), 2.78 (1H, t, $J = 2.7$ Hz, 6-H), 3.33 (3H, s, OCH_3), 3.64 (1H, d, $J = 9.4$ Hz, 24-H), 4.07 (1H, d, $J = 9.1$ Hz, 22-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.1, 13.9, 19.3, 21.5, 21.8, 22.6, 23.3, 23.6, 24.0, 25.0, 26.3, 29.9, 31.6, 33.3, 35.0, 35.2, 41.2, 43.3, 43.4, 47.8, 55.1, 56.6, 56.7, 72.5, 76.2, 78.1, 78.9, 82.3; MS (EI) (rel. int.): 43 (100, base), 464 (0.2, M^+); FABMS (positive) $C_{28}H_{48}O_5$+H: 465.

4.1.7. (20*S*,22*S*,24*S*)-20,22,24,25-Tetrahydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholestane 5. A colorless glass; $[\alpha]_D^{26} = +16.2$ (*c* 0.29, $CHCl_3$); IR: 3420, 2930, 1455, 1380, 1095 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.44 (1H, dd, $J = 7.9$, 4.8 Hz, 4α-H), 0.65 (1H, t, $J = 4.8$ Hz, 4β-H), 0.78–0.90 (3H, m), 0.94 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.11 (3H, s, 21-H_3), 1.03–1.83 (20H, m), 1.18 and 1.23 (each 3H, each s, 26 and 27-H_3), 1.88 (1H, dt, $J = 13.4$, 2.7 Hz, 7-*H*H), 2.12 (1H, dt, $J = 12.5$, 3.0 Hz, 12-*H*H), 2.77 (1H, t, $J = 2.7$ Hz, 6-H), 3.33 (3H, s, OCH_3), 3.64 (1H, d, $J = 9.4$ Hz, 24-H), 4.07 (1H, d, $J = 9.1$ Hz, 22-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.1, 14.2, 19.3, 21.4, 21.5, 22.6, 23.6, 23.7, 24.0, 24.9, 26.5, 29.9, 32.3, 33.3, 35.0, 35.2, 40.7, 43.4, 43.5, 47.9, 55.1, 56.5, 56.6, 72.3, 72.9, 75.0, 78.6, 82.3; MS (CI) (rel. int.): 447 (100, base), 465 (1.1, M^++1); HRMS (CI) calcd for $C_{28}H_{48}O_5$+H: 465.3580; found 465.3557.

4.1.8. (20*R*,22*R*,24*R*)-20,22,24,25-Tetrahydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholestane 6. A colorless glass; $[\alpha]_D^{25} = +38.8$ (*c* 0.52, $CHCl_3$); IR: 3420, 2930, 1455, 1380, 1100 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.44 (1H, dd, $J = 7.9$, 4.8 Hz, 4α-H), 0.65 (1H, t, $J = 4.8$ Hz, 4β-H), 0.79–0.90 (3H, m), 0.93 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.24 (3H, s, 21-H_3), 1.04–1.84 (20H, m), 1.18 and 1.25 (each 3H, each s, 26 and 27-H_3), 1.89 (1H, dt, $J = 13.4$, 2.7 Hz, 7-*H*H), 2.09 (1H, dt, $J = 12.2$, 3.3 Hz, 12-*H*H), 2.77 (1H, br s, 6-H), 3.33 (3H, s, OCH_3), 3.69 (1H, dd, $J = 9.8$, 2.7 Hz, 24-H), 3.77 (1H, dd, $J = 10.1$, 2.4 Hz, 22-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.1, 14.0, 19.3, 20.3, 21.5, 21.9, 22.6, 23.8, 24.0, 25.0, 26.7, 29.9, 32.6, 33.4, 34.9, 35.3, 40.7, 43.4, 43.6, 48.0, 54.8, 56.4, 56.6, 72.6, 72.9, 75.0, 77.5, 82.3; MS (EI) (rel. int.): 43 (100, base), 463 (0.2, M^+-1); HRMS (EI) calcd for $C_{28}H_{48}O_5$–H: 463.3423; found 463.3438.

4.1.9. (20*S*,22*S*,24*R*)-20,22,24,25-Tetrahydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholestane 7. A colorless glass; $[\alpha]_D^{24} = +29.4$ (*c* 1.30, $CHCl_3$); IR: 3425, 2930, 1455, 1380, 1100 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.44 (1H, dd, $J = 7.9$, 4.8 Hz, 4α-H), 0.65 (1H, t, $J = 4.8$ Hz, 4β-H), 0.77–0.91 (3H, m), 0.93 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.10 (3H, s, 21-H_3), 1.04–2.05 (21H, m), 1.18 and 1.23 (each 3H, each s, 26 and 27-H_3), 2.29–2.36 (1H, m), 2.78 (1H, t, $J = 2.7$ Hz, 6-H), 3.32 (3H, s, OCH_3), 3.64 (1H, d, $J = 9.4$ Hz, 24-H), 4.07 (1H, d, $J = 9.1$ Hz, 22-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.1, 13.9, 19.3, 21.5, 21.8, 22.6, 23.3, 23.6, 24.0, 25.0, 26.3, 29.9, 31.6, 33.3, 35.0, 35.2, 41.2, 43.3, 43.4, 47.8, 55.1, 56.6, 56.7, 72.5, 76.2, 78.1, 78.9, 82.3; MS (EI) (rel. int.): 299 (100, base), 464 (0.2, M^+); HRMS (EI) calcd for $C_{28}H_{48}O_5$: 464.3502; found 464.3532.

4.1.10. (20*R*,22*R*)-20,24-Dihydroxy-6β-methoxy-3α,5-*cyclo*-25-homo-5α-cholano-25,22-lactone 13. To a stirred solution of lithium diisopropylamide, prepared from *n*-BuLi (1.59 M in hexane, 0.45 mL, 0.72 mmol) and diisopropylamine (73 mg, 0.72 mmol) in THF (1 mL), was added a solution of lactone **12**[7g] (100 mg, 0.24 mmol) in THF (1 mL) at −78 °C and the mixture stirred at the same temperature for 1 h. To this reaction mixture was added portionwise MoOPH[16] (12 mg, 0.29 mmol) and the mixture stirred at the same temperature for 1.5 h. After the addition of aqueous saturated ammonium chloride, the mixture was allowed to warm to room temperature. Inorganic compounds were filtered off and the filtrate extracted with AcOEt. The extract was washed with aqueous saturated ammonium chloride and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane–AcOEt (65:35, v/v) afforded unreacted lactone **12** (71.7 mg) and an inseparable diastereomeric ca. 1:1 mixture of hydroxy lactone **13** (17.2 mg, 59% based on the recovery of the starting material) as a colorless glass. IR: 3580, 3390, 2920, 1785, 1455, 1380, 1100 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.44 (1H, dd, $J = 7.9$, 4.8 Hz, 4α-H), 0.65 (1H, t, $J = 4.8$ Hz, 4β-H), 0.84–2.23 (21H, m), 0.85 and 0.88 (total 3H, each s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.20 and 1.24 (total 3H, each s, 21-H_3), 2.44–2.58 (1H, m), 2.78 (1H, br s, 6-H), 3.01 and 3.22 (total 1H, each br s, OH), 3.33 (3H, s, OCH_3), 4.38 (0.5H, dd, $J = 10.4$, 6.1 Hz, 22-H), 4.47–4.62 (1.5H, m, 22-H and 24-H); MS (EI) (rel. int.): 43 (100, base), 432 (2.4, M^+); HRMS (EI) calcd for $C_{26}H_{40}O_5$: 432.2874; found 432.2872.

4.1.11. (20*R*,22*R*,24*S*)- and (20*R*,22*R*,24*R*)-20,22,24,25-Tetrahydroxy-6β-methoxy-3α,5-*cyclo*-5α-cholestanes 4 and 6. To a stirred solution of **13** (13.5 mg, 0.03 mmol) in dry THF (1 mL) was added MeMgBr (3 M in Et_2O, 0.1 mL, 0.3 mmol) at −78 °C, and the mixture was allowed to warm to 0 °C. The reaction mixture was stir-

red for 0.5 h at 0 °C. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture then extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane–AcOEt (50:50, v/v) afforded tetraol **4** (6.3 mg, 43%) as a colorless glass. Further elution with hexane–AcOEt (30:70, v/v) afforded tetraol **6** (1.4 mg, 10%) as a colorless glass. Spectroscopic data of both tetraols **4** and **6** obtained by Grignard reaction were identical with those described in the dihydroxylation of (*E*)-20(22),24-cholestadiene **1**.

Acknowledgments

We thank Dr. H. F. Kasai, Dr. M. Shirao, and Dr. A. Shigihara, Hoshi University, for spectral measurements. The financial support of a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan is gratefully acknowledged.

References

1. For recent reviews, see: (a) Dinan, L. *Phytochemistry* **2001**, *57*, 325–339; (b) Dinan, L.; Savchenko, T.; Whiting, P. *Cell. Mol. Life Sci.* **2001**, *58*, 1121–1132; (c) Nakanishi, K. *Steroids* **1992**, *57*, 649–657.
2. For recent reviews, see: (a) Bathori, M.; Pongracz, Z. *Curr. Med. Chem.* **2005**, *12*, 153–172; (b) Lafont, R.; Dinan, L. *J. Insect Sci.* **2003**, *3*, 1–30.
3. For recent reviews, see: (a) Lafont, R.; Dauphin-Villemant, C.; Warren, J. T.; Rees, H. *Compr. Mol. Insect Sci.* **2005**, *3*, 125–195; (b) Dinan, L. *Bioactive Nat. Prod.* **2003**, *29*, 3–71.
4. Pinheiro, M. L. B.; Wolter Filho, W.; Da Rocha, A. I.; Porter, B.; Wenkert, E. *Phytochemistry* **1983**, *22*, 2320–2321.
5. Suksamrarn, A.; Promrangsan, N.; Chitkul, B.; Homvisasevongsa, S.; Sirikate, A. *Phytochemistry* **1997**, *45*, 1149–1152.
6. (a) Suksamrarn, A.; Promrangsan, N.; Jintasirikul, A. *Phytochemistry* **2000**, *53*, 921–924; (b) Coll, J.; Reixach, N.; Sanchez-Baeza, F.; Casas, J.; Camps, F. *Tetrahedron* **1994**, *50*, 7247–7252; (c) Baltaev, U.; Rashkes, Y. V.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1985**, *4*, 522–525; (d) Takemoto, T.; Hikino, Y.; Arai, T.; Kawahara, M.; Konno, C.; Arihara, S.; Hikino, H. *Chem. Pharm. Bull.* **1967**, *15*, 1816.
7. (a) Kametani, T.; Tsubuki, M.; Furuyama, H.; Honda, T. *J. Chem. Soc., Chem. Commun.* **1984**, 375–376; (b) Kametani, T.; Tsubuki, M.; Higurashi, K.; Honda, T. *J. Org. Chem.* **1986**, *51*, 2932–2939; (c) Honda, T.; Keino, K.; Tsubuki, M. *J. Chem. Soc., Chem. Commun.* **1990**, 650–651; (d) Tsubuki, M.; Keino, K.; Honda, T. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2643–2649; (e) Tsubuki, M.; Kanai, K.; Keino, K.; Kakinuma, N.; Honda, T. *J. Org. Chem.* **1992**, *57*, 2930–2934; (f) Tsubuki, M.; Takada, H.; Katoh, T.; Miki, S.; Honda, T. *Tetrahedron* **1996**, *46*, 14515–14532; (g) Tsubuki, M.; Ohinata, A.; Tanaka, T.; Takahashi, K.; Honda, T. *Tetrahedron* **2005**, *61*, 1095–1100.
8. For recent reviews, see: (a) Kolb, H. C.; Sharpless, K. B.. In *Transition Metals for Organic Synthesis*; Beller, M., Bolm, C., Eds.; Wiley-VCH: Weinheim, 2004; Vol. 2, pp 275–298; (b) Becker, H.; Sharpless, K. B. In *Asymmetric Oxidation Reactions*; Katsuki, T., Ed.; Oxford University Press: Oxford, 2001; pp 81–104; (c) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483–2547.
9. Suksamrarn et al. reported the synthesis of 24-*epi*-abutasterone by the asymmetric dihydroxylation of stachysteorne C. Yingyongnarongkul, B.-E.; Suksamrarn, A. *Tetrahedron* **1998**, *54*, 2795–2800.
10. (a) Bannai, K.; Morisaki, M.; Ikekawa, N. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2116–2120; (b) Morisaki, M.; Sato, S.; Ikekawa, N. *Chem. Pharm. Bull.* **1977**, *25*, 2576–2583.
11. Byon, C.-Y.; Gut, M. *J. Org. Chem.* **1976**, *41*, 3716–3722.
12. (a) Ansell, M. F.; Thomas, D. A. *J. Chem. Soc.* **1961**, 539–542; (b) Büchi, G.; Powell, J. E., Jr. *J. Am. Chem. Soc.* **1970**, *92*, 3126–3133; (c) Jiang, W.; Fuertes, M. J.; Wulff, W. D. *Tetrahedron* **2000**, *56*, 2183–2194.
13. (a) Schmidt, J. P.; Piraux, M.; Pilette, J. F. *J. Org. Chem.* **1975**, *40*, 1586–1588; (b) Schow, S. R.; McMorris, T. C. *J. Org. Chem.* **1979**, *44*, 3760–3765.
14. (a) Bates, R. B.; Carnighan, R. H.; Rakutis, R. O.; Schauble, J. H. *Chem. Ind.* **1962**, 1020–1021; (b) Trost, B. M. *Acc. Chem. Res.* **1970**, *3*, 120–130.
15. See, for example: (a) Cossy, J.; Pradaux, F.; BouzBouz, S. *Org. Lett.* **2001**, *3*, 2233–2235; (b) Miyashita, K.; Ikejiri, M.; Kawasaki, H.; Maemura, S.; Imanishi, T. *Chem. Commun.* **2002**, 742–743; (c) Reddy, Y. K.; Falck, J. R. *Org. Lett.* **2002**, *4*, 969–971.
16. (a) Vedejs, E. *J. Am. Chem. Soc.* **1974**, *96*, 5944–5946; (b) Vedejs, E.; Engler, D. A.; Telschow, J. E. *J. Org. Chem.* **1978**, *43*, 188–196.
17. (a) Corey, E. J.; Grogan, M. J. *Tetrahedron Lett.* **1998**, *39*, 9351–9354; (b) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Oliver, B. B.; Su, J.-L.; Sundseth, S. S.; Winegar, D. A.; Blanchard, D. E.; Spencer, T. A.; Wilson, T. M. *J. Biol. Chem.* **1997**, *272*, 3137–3140; (c) Janowski, B. A.; Willey, P. J.; Devi, T. R.; Falck, J. R.; Mangelsdorf, D. J. *Nature* **1996**, *383*, 728–731.