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Configuration at the C-23 Position of 23-Hydroxy- and 23,25-Dihydroxycholesterols

YUTAKA HIRANO, TADASHI EGUCHI, MASAJI ISHIGURO,[1] and NOBUO IKEKAWA*

Department of Chemistry, Tokyo Institute of Technology, Ohokayama, Meguro-ku, Tokyo 152, Japan

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(23*R*)- and (23*S*)-23-Hydroxy- and 23,25-dihydroxycholesterols were synthesized *via* 3β-tetrahydropyranyloxycholesta-5,25-dien-23-ol (**5**) from cholenic acid acetate (**1**). The stereochemistry at the C-23 position of related steroids is also discussed.

Keywords——23-hydroxycholesterol; 23,25-dihydroxycholesterol; determination of stereochemistry; steroidal side chain

Recently, various 23-hydroxylated steroids, such as 23,25-dihydroxyvitamin D_3,[2] calcidiol lactone[3] (vitamin D_3 metabolites), antheridiol (fungal sex hormone),[4] and brassinolide (plant growth promoter),[5] have been isolated and their structures determined by direct comparison with synthetic samples or by X-ray crystallography. However, the configurations at C-23 of chiograsterol,[6] 23-hydroxylanosterol,[7] 23-hydroxycholesterol sulfate,[8] and several bile alcohols[9] are still obscure, because their C-23 stereochemistries were assigned only on the basis of molecular rotations. Therefore, in order to compare their molecular rotations and other physical properties with those of the natural 23-hydroxylated steroids, (23*R*)- and (23*S*)-hydroxy- and 23,25-dihydroxycholesterols were synthesized by unequivocal procedures.[10]

Cholenic acid acetate (**1**) was converted to the 22-olefin **2a** by oxidative decarboxylation[11] with lead tetraacetate, cupric acetate and pyridine in refluxing benzene in 61% yield. After the protecting group of the 3-hydroxyl group had been changed to tetrahydropyranyl ether, the 22-olefin **2c** was treated with BH_3–tetrahydrofuran complex and then with alkaline hydrogen peroxide to give the 23-alcohol **3**. Oxidation of **3** with pyridinium chlorochromate in methylene chloride in the presence of sodium acetate afforded the 23-aldehyde **4** in 41% yield from **2a**. When the 23-aldehyde **4** was coupled with methallyl chloride in tetrahydrofuran by means of the Grignard reaction, the 25-en-23-alcohol **5** was obtained in 96% yield as a 1 : 1 mixture of 23-epimers. In order to separate the C-23 epimers, this product was converted to the 3,23-dibenzoate, which was crystallized from acetone to give the more polar dibenzoate **6a**. The mother liquor was concentrated and the resulting residue was hydrolyzed to the 3,23-diol, which was crystallized from petroleum ether–ether to afford the fraction enriched with the diol **7b** whose dibenzoate **7a** was less polar than **6a**. Rebenzoylation followed by preparative thin layer chromatography afforded the less polar dibenzoate **7a** in a pure form. From the dibenzoates **6a** and **7a**, the 3,23-diols **6b** and **7b** were obtained, respectively. Oxymercuration of the 25-olefin-3,23-dibenzoate **6a** with $Hg(OAc)_2$ followed by reduction with $NaBH_4$ gave the 25-hydroxy-3,23-dibenzoate **8a**, which was hydrolyzed with KOH–MeOH to give the more polar 3,23,25-triol **8b**. The configuration at the C-23 position of **8b** was determined as 23*S*(β_F) by X-ray crystallography as reported in our previous paper.[10] By the same procedure, the 25-olefin-3,23-dibenzoate **7a** gave the 25-hydroxy-3,23-dibenzoate **9a** and the less polar 3,23,25-triol **9b**, both of which should have 23*R*(α_F-configuration. Hence, the C-23 configurations of the 25-olefins **6** and **7** were also determined as *S* and *R*, respectively. Catalytic hydrogenation on Pt in methanol of the (23*S*)-dienol **6b** and (23*R*)-dienol **7b** afforded authentic samples of (23*R*)-23-hydroxycholesterol (**10a**) and (23*S*)-23-hydroxycholesterol (**11a**),

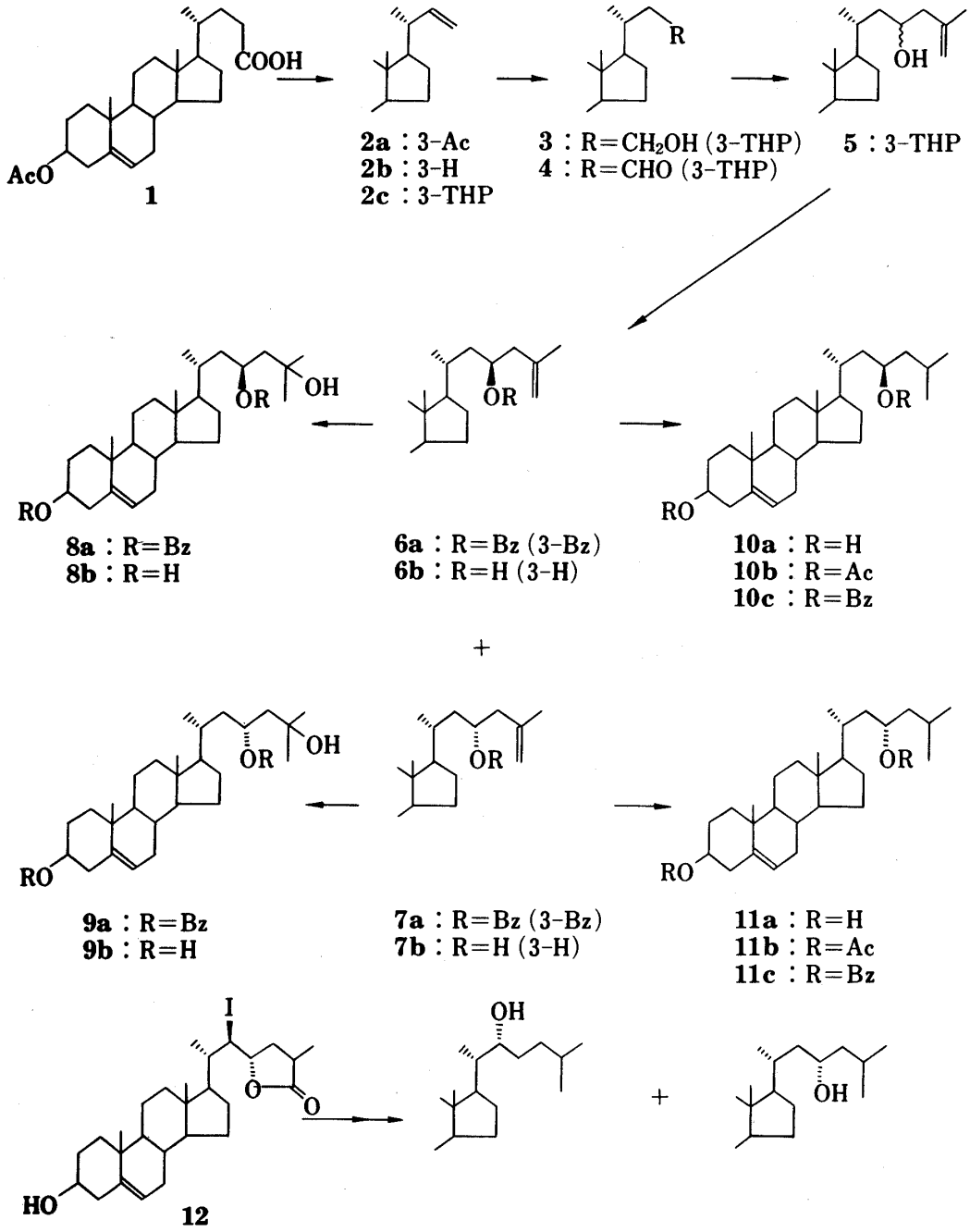

Chart 1

respectively. Thus, the chromatographically pure (23*R*)- and (23*S*)-epimers of 23,25-dihydroxy- and 23-hydroxycholesterols were obtained.

Physical poperties of 23-hydroxy- and 23,25-dihydroxycholesterols and some ester derivatives are listed in Table I. A pair of C-23 epimers of 23-hydroxylated sterols could be separated either in the form of the free alcohols (**6b** and **7b**; **8b** and **9b**; **10a** and **11a**) or of their dibenzoates (**6a** and **7a**; **8a** and **9a**; **10c** and **11c**) by thin layer chromatography using multiple developments as well as high performance liquid chromatography employing a normal phase column.[12] In the case of 3,23-dibenzoate derivatives of 23-hydroxylated sterols, diagnostic differences can be noted in the chemical shift of the C-18 methyl signal in the ^{1}H-NMR spectra; 0.70—0.72 ppm for the 23-β_F series (**6a**, **8a** and **10c**); 0.62 for the 23-α_F series (**7a**, **9a** and **11c**). Lier and Smith[13] prepared both epimers of 23-hydroxycholesterol by reduction of 23-oxocholesterol. They concluded that the less polar and more dextrorotatory isomer has

TABLE I. Physical Properties of 23-Hydroxy- and 23,25-Dihydroxycholesterols

Compound	mp (°C)	$[\alpha]_D$
(23*R*)-23-Hydroxycholesterol (**10a**)	163—166 (175—176)[a]	0° (−22°)[a]
(23*S*)-23-Hydroxycholesterol (**11a**)	160—162 (136—137)[a]	−35.7° (−30°)[a]
(23*R*)-23-Hydroxycholesterol 3,23-diacetate (**10b**)	104.5—106 (111—114)[a]	0.1° (−9°)[a]
(23*S*)-23-Hydroxycholesterol 3,23-diacetate (**11b**)	144.5—146 (143—146)[a]	−41.4° (−35°)[a]
(23*R*)-23-Hydroxycholesterol 3,23-dibenzoate (**10c**)	171—172	0.8°
(23*S*)-23-Hydroxycholesterol 3,23-dibenzoate (**11c**)	161—162	3.7°
(23*R*)-23,25-Dihydroxycholesterol (**8b**)	218—220	−2.2°
(23*S*)-23,25-Dihydroxycholesterol (**9b**)	205—207	−12.9°
(23*R*)-23,25-Dihydroxycholesterol 3,23-dibenzoate (**8a**)	221—222	−7.1°
(23*S*)-23,25-Dihydroxycholesterol 3,23-dibenzoate (**9a**)	176—177	+9.2°

a) Reported by Lier and Smith.[13]

23*S*(α_F)- and the more polar isomer has 23*R*(β_F)-configuration by analogy with the rotation data for 23-hydroxylanosterol. Their conclusion is consistent with ours, although significant differences in the physical properties were observed, as shown in Table I, probably due to the presence of impurities in their samples.

It should be noted that the previous assignment[14] of the stereochemistry of the iodolactone **12**, a synthetic progenitor of the calcidiol lactone,[3] has now been confirmed by the present work. Thus, the 23-hydroxycholesterol derived from **12** by a stereochemically defined process was found to be identical with (23*S*)-23-hydroxycholesterol in respect of the retention time on high performance liquid chromatography.

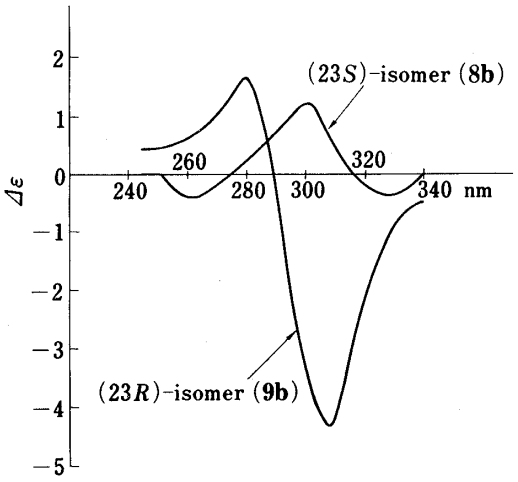

Fig. 1. Circular Dichroism of (23*R*)- and (23*S*)-23, 25-Dihydroxycholesterols with Eu(fod) in Dry $CHCl_3$

Recently, Dayal *et al.*[9a] and Hoshita *et al.*[9b] have independently identified a 23-hydroxylated bile alcohol, 5β-cholestane-3α,7α,12α, 23,25-pentol, in patients with cerebrotendinous xanthomatosis. Although the samples of the two research groups seem to be identical based on the mp and $[\alpha]_D$values, Hoshita *et al.*[9b] assigned the configuration at the C-23 position of the pentol as *S* by comparing its molecular rotation with those of epimeric 23-hydroxylanosterols, whereas Dayal *et al.*[9c] claimed it to be 23*R* based on the results of circular dichroism spectroscopy using $Eu(fod)_3$.[15] Table II summarizes the reported physical data for the natural 3α,7α,12α,23,25-pentol and 5β-cholestane-3α,7α,12α,23-tetrol isolated and synthesized by Hoshita *et al.*[9d,e] together with those for the presently prepared (23*R*)- and (23*S*)-epimers of 23,25-dihydroxy-cholesterols and 23-hydroxycholesterols. It can be seen that in all cases the β_F-epimers are more dextrorotatory and more polar than the α_F-counterparts. In the case of 23,25-dihydroxysterols, the natural 3α,7α,12α,23,25-pentol and (23*S*)-23,25-dihydroxycholesterol (**8b**) have more positive molecular rotation values than the corresponding 23-deoxysterols, 5β-cholestane-3α,7α,12α,25-tetrol[9b] and 25-hydroxycholesterol,[16] respectively. These observations support the view that the natural 3α,7α,12α,23,25-pentol isolated by Hoshita *et al.* has 23*S*-configuration. The opposite assignment obtained by Dayal *et al.* can be explained by assuming that the sign of the CD spectrum of this particular pentol is

TABLE II. Physical Properties of Hydroxysteroids

Sterol	M_D	Polarity on SiO_2 TLC	Sign on CD
5β-Cholestane-3α,7α,12α,23,25-pentol (natural)	+222[9b]	More polar[9a]	−[9c]
5β-Cholestane-3α,7α,12α,25-tetrol	+166[9b]		
(23*R*/α$_F$)-23,25-Dihydroxycholesterol(**9b**)	−54	Less polar	−
(23*S*/β$_F$)-23,25-Dihydroxycholesterol(**8b**)	−9	More polar	+
25-Hydroxycholesterol	−37[16]		
(23*S*/α$_F$)-5β-Cholestane-3α,7α,12α,23-tetrol (synthetic)	+179[9e]	Less polar[9e]	
(23*R*/β$_F$)-5β-Cholestane-3α,7α,12α,23-tetrol (natural)	+227[9e]	More polar[9e]	
(23*S*/α$_F$)-23-Hydroxycholesterol (**11a**)	−143	Less polar	
(23*R*/β$_F$)-23-Hydroxycholesterol (**10a**)	0	More polar	

opposite to that of 23,25-dihydroxycholesterols or that Dayal's sample is the C-23 epimer of Hoshita's sample.

Experimental

Melting points were determined on a hot stage microscope and are uncorrected. Optical rotations were taken in chloroform solution on a JASCO-DIP-S polarimeter, unless otherwise noted. ^{1}H-NMR spectra were recorded on a Hitachi R-24A or JEOL JNM-PS-100 spectrometer in deuteriochloroform solution, unless otherwise noted, with tetramethylsilane as an internal reference. Mass spectra were obtained with a Shimadzu LKB-9000S instrument. High performance liquid chromatography (HPLC) was carried out with a Shimadzu LC-3A liquid chromatograph with a Shimadzu SPD-1 spectrophotometric detector, using a Zorbax SIL normal phase column (15 cm×4.6 mm i.d.). Column chromatography was effected with Merck Kieselgel 60 and thin layer chromatography was carried out on Merck precoated Kieselgel 60 F_{254} (0.25 or 0.5 mm thickness). The circular dichroism (CD) measurements were carried out on a Jasco J-500C spectropolarimeter with a 0.1 cm cell using a 1:1 mixture of the steroid and the complex Eu(fod)$_3$ [tris (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionate) europium] in dry chloroform (ethanol-free). The concentration of the solutes was 2×10^{-4} M. "The usual work-up" refers to dilution with water, extraction with the organic solvent indicated in parentheses, washing of the extract to neutrality, drying over $MgSO_4$, filtration and removal of the solvent by evaporation under a vacuum.

The following abbreviations are used: THF, tetrahydrofran; THP, tetrahydropyranyl; s, singlet; d, doublet; dd, double doublet; m, multiplet; br, broad.

24-Norchola-5,22-dien-3β-ol Acetate (2a)——A mixture of cholenic acid acetate (**1**) (7.8 g), cupric acetate (0.7 g), lead tetraacetate (13.9 g) and pyridine (0.56 ml) in dry benzene (300 ml) was stirred at reflux for 3 h under argon. After the mixture had been cooled to room temperature, ethylene glycol (25 ml) was added and the whole was stirred for 10 min. The usual work-up (ether) gave an oily residue which was chromatographed. Elution with benzene gave the 22-olefin **2a** (4.19 g), mp 126—126.5 °C (methanol) (lit.[17] 122—124 °C); NMR, δ 0.70 (3H, s, 18-H$_3$), 1.00 (3H, s, 19-H$_3$), 1.02 (3H, d, *J*=6 Hz, 21-H$_3$), 1.99 (3H, s, OAc) 4.54 (1H, m, 3-H), 4.68 (1H, d, *J*=2.2 Hz, 23-H), 4.89 (1H, d.d, *J*=8 and 2.2 Hz, 23-H), 5.32 (1H, m, 6-H), 5.72 (1H, dd, *J*=16 and 8 Hz, 22-H). *Anal.* Calcd for $C_{25}H_{38}O_2$ C, 81.03; H, 10.34. Found: C, 81.17; H, 10.37.

Further elution with benzene–ethyl acetate (1:2) afforded the starting material **1** (2.66 g).

24-Norchola-5,22-dien-3b-ol (2b)——A solution of the 22-olefin **2a** (7.47 g) and 5% methanolic KOH (30 ml) in THF (30 ml) was stirred at room temperature for 16 h. The usual work-up (ethyl acetate) yielded the 3-alcohol **2b** (6.6 g), mp 121—123 °C (methanol); NMR δ 0.70 (3H, s, 18-H$_3$), 1.01 (3H, s, 19-H$_3$), 1.03 (3H, d, *J*=6 Hz, 21-H$_3$), 3.50 (1H, m, 3-H), 4.68 (1H, d, *J*=2.2 Hz 23-H), 4.89 (1H, dd, *J*=8 and 2.2 Hz, 23-H), 5.32 (1H, m, 6-H), 5.72 (1H, dd., *J*=16 and 8 Hz, 22-H). *Anal.* Calcd for $C_{23}H_{36}O$: C, 84.06; H, 11.05. Found: C, 83.76; H, 11.05.

24-Norchola-5,22-dien-3β-ol THP Ether (2c)——The alcohol **2b** (6.6 g) was treated with dihydropyran (2.6 ml) and a catalytic amount of *p*-toluenesulfonic acid·H_2O in methylene chloride (20 ml) at room temperature for 30 min. The usual work-up (ether) followed by chromatography with benzene as the eluent yielded the 3-THP ether **2c** (7.52 g), mp 127—129 °C (acetone); NMR δ 0.70 (3H, s, 18-H$_3$), 1.01 (3H, s, 19-H$_3$), 3.20—4.10 (3H, m, 3-H and 6′-H$_2$ of THP), 4.68 (2H, m, 23-H and 2′-H of THP), 4.87 (1H, dd, *J*=10.4 and 2.4 Hz, 23-H), 5.32 (1H, m, 6-H), 5.70 (1H, dd, *J*=16 and 10.4 Hz, 22-H). *Anal.* Calcd for $C_{28}H_{44}O_2$: C, 81.50; H, 10.75. Found: C, 80.98; H, 10.77.

24-Norchol-5-ene-3β,23-diol 3-THP Ether (3)——BH_3–THF complex (1 M, 13 ml) was added to a solution of the 22-olefin **2c** (4.39 g) in dry THF (100 ml) at 0 °C over 2 h under argon. The mixture was stirred for 30 min,

then 3N sodium hydroxide (13 ml) and 30% hydrogen peroxide solution (13 ml) were added in that order and the whole was stirred at 0 °C for 30 min. The usual work-up (ethyl acetate) and chromatography with benzene–ethyl acetate (5:1) as the eluent gave the 23-alcohol **3** (3.28 g), mp 139—143 (acetone); NMR δ 0.69 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 3.20—4.30 (5H, m, 3-H, 23-H_2, and 6'-H_2 of THP), 4.68 (1H, m, 2'-H of THP), 5.30 (1H, m, 6-H). *Anal.* Calcd for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 78.24; H, 11.09.

Cholesta-5,22-diene-3β,23-diol 3-THP Ether (5)——A solution of the 23-alcohol **3** (3.28 g) in methylene chloride (25 ml) was added in one portion to a suspension of pyridinium chlorochromate (3.2 g) and sodium acetate (240 mg) in methylene chloride (25 ml). The mixture was stirred for 2 hr, ether (300 ml) was added, and the whole was passed through a pad of Florisil (60 g) and eluted with ether. The solvent was evaporated off and the residue was chromatographed on silica gel. Elution with benzene–ethyl acetate (100:1) afforded the 23-aldehyde **4** (2.06 g); NMR δ 0.70 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 1.03 (3H, d, J=6 Hz, 21-H_3), 3.20—4.30 (3H, m, 3-H and 6'-H_2 of THP), 4.70 (1H, m, 2'-H of THP), 5.32 (1H, m, 6-H), 9.70 (1H, m, CHO).

Methally chloride (4.7 ml) was added dropwise to a suspension of magnesium (4.7 g) in dry THF (20 ml) at 0 °C over 1 h and the mixture was stirred for 40 min. Then, a solution of the 22-aldehyde **4** (3.0 g) in dry THF (20 ml) was added and the whole was stirred at 0 °C for 1 h under argon. The reaction was quenched with saturated ammonium chloride (30 ml). The usual work-up (ether) gave a 1:1 mixture of the epimeric 23-alcohols **5** (3.17 g); NMR δ 0.70 (3H, s, 18-H_3), 1.00 (3H, s, 19-H_3), 1.74 (3H, s, 27-H_3), 3.20—4.10 (4H, m, 3-H, 23-H and 6'-H_2 of THP), 4.50—5.00 (3H, m, 26-H_2 and 2'-H of THP), 5.32 (1H, m, 6-H).

(23*S* and 23*R*)-Cholesta-5,25-diene-3β,23-diol Dibenzoate (6a and 7a)——A solution of the 23-alcohol **5** (3.17 g) in methanol–THF (5:1, 12 ml) containing a few drops of 2N HCl solution was stirred at room temperature for 2 h. The usual work-up (ethyl acetate) gave the 3,23-diol (2.62 g), which was treated with benzoyl chloride (1.83 ml) and pyridine (10 ml) at 0 °C for 30 min. The usual work-up (ether) yielded the 3,23-dibenzoate (3.56 g). Crystallization from acetone afforded the more polar 3,23*S*-dibenzoate **6a** (1.28 g), mp 151—153 °C; $[\alpha]_D^{25}$ −17.7° (c=1.0); TLC Rf=0.56 (hexane–benzene, 1:1, developed four times); NMR δ 0.70 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.76 (3H, s, 27-H_3), 4.72 (2H, brs, 26-H_2),4.80 (1H, m, 3-H), 5.35 (2H, m, 6-H and 23-H), 7.20—7.70 and 7.80—8.20 (10H, m, aromatic). *Anal.* Calcd for $C_{41}H_{52}O_4$: C, 80.88; H, 8.61. Found: C, 80.52; H, 8.48.

The mother liquor was concentrated and the residue (1.34 g) was hydrolyzed with 5% methanolic potassium hydroxide (10 ml) in THF (5 ml) at room temperature for 16 h. The resulting 3,23-diol (0.82 g) was crystallized from petr. ether–ether to afford the fraction enriched with the less polar 3,23*R*-diol **7b** (482 mg), which was re-benzoylated and then purified by preparative TLC (benzene–hexane, 1:1; developed five times) to give the less polar 3,23*R*-dibenzoate **7a** (670 mg) as an amorphous solid $[\alpha]_D^{25}$ +5.49° (c=1.0); TLC Rf=0.60 (hexane–benzene, 1:1, developed four times); NMR δ 0.62 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.79 (3H, s, 27-H_3), 4.72 (2H, br s, 26-H_2), 4.80 (1H, m, 3-H), 5.35 (2H, m, 6-H and 23-H), 7.20—7.70 and 7.80—8.20 (10H, m, aromatic). The 3,23-dibenzoates were analyzed by HPLC (solvent system, hexane–methylene chloride, 5:1). The retention times of (23*S*)-(**6a**) and the (23*R*)-isomer (**7a**) were 3.8 min and 3.4 min, respectively.

(23*S* and 23*R*)-Cholesta-5,25-diene-3β,23-diol (6b and 7b)——The more polar 3,23*S*-dibenzoate **6a** (1.08 g) was hydrolyzed with 5% methanolic potassium hydroxide (10 ml) in THF (5 ml) at room temperature for 16 h. The usual work-up (ethyl acetate) gave the more polar 3,23S-diol **6b** (850 mg), mp 153—154.5 °C (acetone), $[\alpha]_D^{25}$ −30.2° (c=1.0); TLC Rf=0.34 (solvent system, benzene–ethyl acetate, 5:1, developed twice); NMR δ 0.70 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 1.76 (3H, s, 27-H_3), 3.20—4.00 (2H, m, 3- and 23-H), 4.65—5.00 (2H, m, 26-H_2), 5.30 (1H, m, 6-H). *Anal.* Calcd for $C_{27}H_{44}O_2$: C, 80.94; H, 11.70. Found: C, 81.10; H, 11.51.

The less polar 3,23*R*-diol **7b** (430 mg) was obtained from the less polar 3,23*R*-dibenzoate **7a** (650 mg) in the same manner, mp 126—128.5 °C (ether–hexane), $[\alpha]_D^{25}$ −31.0° (c=1.0); TLC Rf=0.38 (solvent system, benzene–hexane, 5:1, developed twice); NMR δ 0.70 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 1.76 (3H, s, 27-H_3), 3.20—4.00 (2H, m, 3- and 23-H), 4.65—5.00 (2H, m, 26-H_2), 5.30 (1H, m, 6-H). *Anal.* Calcd for $C_{27}H_{44}O_2$: C, 80.94; H, 11.70. Found: C, 80.95; H, 11.30.

(23*S* and 23*R*)-Cholest-5-ene-3β,23,25-triol 3,23-Dibenzoate (8a and 9a)——A solution of the more polar olefin **6a** (150 mg) in THF (0.6 ml) was added dropwise to a mixture of mercuric acetate (94 mg), water (0.9 ml) and THF (0.3 ml) at 0 °C. The whole was stirred at room temperature for 16 h. A 3N NaOH solution (0.1 ml) and then a suspension of $NaBH_4$ (300 mg) in 3N NaOH (1.5 ml) were added. The resulting Hg was filtered off and the filtrate was extracted with ethyl acetate. The usual work-up gave a crude product (154 mg), which was chromatographed. Elution with benzene recovered the starting material (51 mg). Further elution with benzene–ethyl acetate (50:1) gave the less polar 25-alcohol **8a** (83 mg), mp 221—222 °C (acetone–hexane); $[\alpha]_D^{28}$ −7.1° (c=0.67); TLC Rf=0.45 (solvent system, hexane–ethyl acetate, 5:1, developed four times); NMR δ 0.72 (3H, s, 18-H_3), 1.07 (3H, s, 19-H_3), 1.27 (6H, s, 26-H_3 and 27-H_3), 4.87 (1H, m, 3-H), 5.37 (1H, m, 23-H), 5.41 (1H, m, 6-H), 7.30—7.70 and 7.90—8.20 (10H, m, aromatic).

The less polar olefin **7a** (120 mg) was converted to the more polar 25-alcohol **9a** (56 mg) in the same manner as described for **6a**; mp 176—177 °C (acetone–hexane); $[\alpha]_D^{28}$ +9.2° (c=0.79); TLC Rf=0.41 (solvent system, hexane–ethyl acetate, 5:1, developed four times). NMR δ 0.62 (3H, s, 18-H_3), 1.05 (3H, s, 19-H_3), 1.20 and 1.25 (3H×2, a pair of s, 26-H_3 and 27-H_3), 4.84 (1H, m, 3-H), 5.41 (1H, m, 6-H), 5.48 (1H, m, 23-H), 7.30—7.75

and 7.90—8.30 (10H, m, aromatic).

The 3,23-dibenzoates were analyzed by HPLC (solvent system, hexane: methylene chloride = 2:1). The retention times of (23*S*)- (**8a**) and the (23*R*)-isomer (**9a**) were 5.2 min and 5.6 min, respectively.

(23*S* and 23*R*)-Cholest-5-ene-3,23,25-triol (8a and 9a)———The less polar dibenzoate **8a** (69 mg) was hydrolyzed with 5% methanolic KOH (10 ml) and THF (6 ml) to give the more polar triol **8b** (45 mg), mp 218—220 °C (dec.) (methanol-water); $[\alpha]_D^{28}$ −2.2° (*c*=0.2, EtOH), TLC *Rf*=0.30 (solvent system, hexane-ethyl acetate, 1:1, developed twice); NMR ($CDCl_3+CD_3OD$) δ 0.70 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.25 and 1.29 (3H×2, a pair of s, 26-H_3), and 27-H_3), 3.40 (1H, m, 3-H), 4.06 (1H, m, 23-H), 5.32 (1H, m, 6-H); MS *m/z*: 418 (M^+). The configuration at C-23 of **8b** was established as 23*S* by X-ray crystallography.[10]

The more polar dibenzoate **9a** (24 mg) was converted to the less polar triol **9b** (16 mg) in the same way, mp 205—207 °C (methanol-water), $[\alpha]_D^{28}$ −12.9° (*c*=0.5, EtOH), TLC *Rf*=0.32 (solvent system, hexane-ethyl acetate, 1:1, developed twice); NMR ($CDCl_3+CD_3OD$) δ 0.72 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 1.25 and 1.31 (3H×2, a pair of s, 26-H_3 and 27-H_3), 3.48 (1H, m, 3-H), 4.13 (1H, m, 23-H),5.36 (1H, m, 6-H); MS *m/z*: 418 (M^+).

(23*R* and 23*S*)-Cholest-5-ene-3β,23-diol (10a and 11a)———A mixture of the more polar 25-olefin **6b** (65 mg) and platinum black, prepared from platinum oxide (20 mg) in methanol (2 ml) was stirred at room temperature for 30 min under a hydrogen atmosphere. The catalyst was removed by filtration and the filtrate was concentrated to give the more polar 3,23*R*-diol **10a** (64 mg), mp 163—166 °C (methanol); $[\alpha]_D^{28}$ 0° (*c*=1.0), NMR δ 0.70 (3H, s, 18-H_3), 0.92 (6H, d, *J*=7 Hz, 26-H_3 and 27-H_3), 1.01 (3H, s, 19-H_3), 3.30—3.94 (2H, m, 3-H and 23-H), 5.34 (1H, m, 6-H). *Anal.* Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.51. Found: C, 80.66; H, 11.70.

The less polar 3,23*S*-diol **11a** (48 mg) was obtained from the less polar 25-olefin **7b** (50 mg) in the same manner, mp 160—162 °C (methanol), $[\alpha]_D^{28}$ −35.7° (*c*=1.0); NMR δ 0.71 (3H, s, 18-H_3), 0.91 (6H, d, *J*=7 Hz, 26-H_3 and 27-H_3), 1.00 (3H, s, 19-H_3), 3.30—3.92 (2H, m, 3-H and 23-H), 5.32 (1H, m, 6-H). *Anal.* Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 81.27; H, 11.60.

(23*R* and 23*S*)-Cholest-5-ene-3β,23-diol Diacetate (10b and 11b)———The 3,23*R*-diol **10a** (70 mg) was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) to afford the more polar diacetate **10b** (83 mg), mp 104.5—106 °C (petr. ether-ether), $[\alpha]_D^{28}$ −0.1° (*c*=1.0); NMR δ 0.69 (3H, s, 18-H_3), 0.90 (6H, d, *J*=6 Hz, 26-H_3, and 27-H_3), 1.01 (3H, m, 19-H_3), 2.02 (6H, s, 3- and 23-OAc), 4.62 (1H, m, 3-H), 5.00 (1H, m, 23-H), 5.34 (1H, m, 6-H).

The less polar 3,23*S*-diacetate **11b** (70 mg) was obtained from the less polar 3,23*S*-diol **11a** (60 mg) in the same manner, mp 144.5—146 °C (methanol); $[\alpha]_D^{28}$ −41.4° (*c*=1.0); NMR δ 0.67 (3H, s, 18-H_3), 0.90 (6H, d, *J*=6 Hz, 26-H_3 and 27-H_3), 1.01 (3H, s, 18-H_3), 2.01 (6H, s, 3- and 23-OAc), 4.60 (1H, m, 3-H), 5.10 (1H, m, 23-H), 5.36 (1H, m, 6-H).

(23*R* and 23*S*)-Cholest-5-ene-3β,23-diol Dibenzoate (10c and 11c)———The 3,23*R*-diol **10a** (82 mg) was benzoylated with benzoyl chloride (60 μl) and pyridine (1 ml) to give the more polar 3,23*R*-dibenzoate **10c** (100 mg), mp 171—172 °C (acetone-methanol); $[\alpha]_D^{25}$ +0.8° (*c*=1.0); NMR δ 0.71 (3H, s, 18-H_3), 0.93 (6H, d, *J*=6 Hz, 26-H_3 and 27-H_3), 1.05 (3H, s, 19-H_3), 4.78 (1H, m, 3-H), 5.27 (1H, m, 23-H), 5.40 (1H, m, 6-H), 7.30—7.70 and 7.80—8.20 (10H, m, aromatic). *Anal.* Calcd for $C_{41}H_{54}O_4$: C, 80.61; H, 8.91. Found: C, 80.51; H, 9.00.

The less polar 3,23*S*-dibenzoate **11c** (36 mg) was obtained from the 3,23*S*-diol **11a** (29 mg) in the same manner, mp 161—162 (acetone-methanol); $[\alpha]_D^{25}$ +3.7° (*c*=1.0); NMR δ 0.62 (3H, s, 18-H_3), 1.00 (6H, d, *J*=6 Hz, 26-H_3 and 27-H_3), 1.04 (3H, s, 19-H_3), 4.87 (1H, m, 3-H), 5.36 (1H, m, 23-H), 5.41 (1H, m, 6-H), 7.30—7.70 and 7.80—8.20 (10H, m, aromatic). *Anal.* Calcd for $C_{41}H_{54}O_4$: C, 80.61; H, 8.91. Found: C, 80.77; H, 8.89.

The 3,23-dibenzoates were analyzed by HPLC (solvent system, hexane: methylene chloride=5:1). The retention times of (23*R*)-(**10c**) and the (23*S*)-isomer (**11c**) were 3.6 and 3.0 min, respectively.

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