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Studies on Steroidal Plant-Growth Regulator 25. Concise Stereoselective Construction of Sidechain of Brassinosteroid from the Intact Sidechain of Hyodeoxycholic Acid: Formal Syntheses of Brassinolide, 25-Methylbrassinolide, 26,27-Bisnorbrassinolide and their Related Compounds

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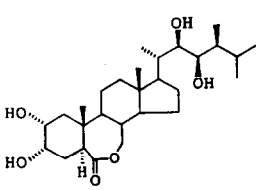
Abstract: A concise stereoselective construction of sidechain of brassinolide (**1**), 25-methylbrassinolide (**3**) and 26,27-bisnorbrassinolide (**4**), which involves β-alkylative 1,3- carbonyl transposition of the α,β-unsaturated ketones **11** and **34** and α,β-unsaturated methyl ester **26** , using the intact sidechain of hyodeoxycholic acid (**2**) as starting material, is described. The formal syntheses of 1 , 3 and 4 were accomplished. In the mean time, 25-methylcastasterone (**21**), 26,27-bisnortyphasterol(**6**) and the new 25-methyltyphasterol (**5**) were also synthesized.

Since the discovery of brassinolide (**1**) as a plant growth promoting steroid [1], a number of attempts have been made to find an efficient method for preparation of brassinolide and its analogues, inter alia, for construction of their sidechain.[2] Although the hyodeoxycholic acid (**2**) is a selected starting material for construction of the sidechain of brassinosteroid[3], its carboxyl side chain must be at first degraded to 20-carbaldehyde, involving multistep manipulation and procedure in decarboxylation with $Pb(OAc)_4$ with a lot of trouble[4]. On this account, we planned to utilize directly the intact sidechain of hyodeoxycholic acid along with the reaction of β-alkylative 1,3-carbonyl transposition[5] for construction of various sidechains of brassinosteroid.



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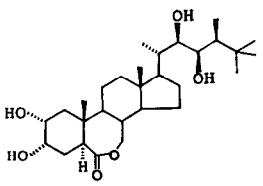

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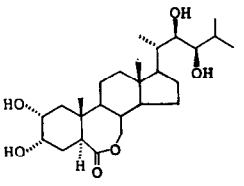

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Recently, two brassinolide analogues, 25-methylbrassinolide (3)[6] which was proved to be more potent than brassinolide and 26,27-bisnorbrassinolide (4)[7] which has almost the same activity as brassinolide, have been synthesized by Mori[6] and Ikekawa[7a], respectively. In the present paper we have used our improved method for construction of side-chain of brassinosteroid. Thus, brassinolide (1), 25-methylbrassinolide (3), 26,27-bisnorbrassinolide (4) and their related compounds were synthesized (Scheme 1-3).

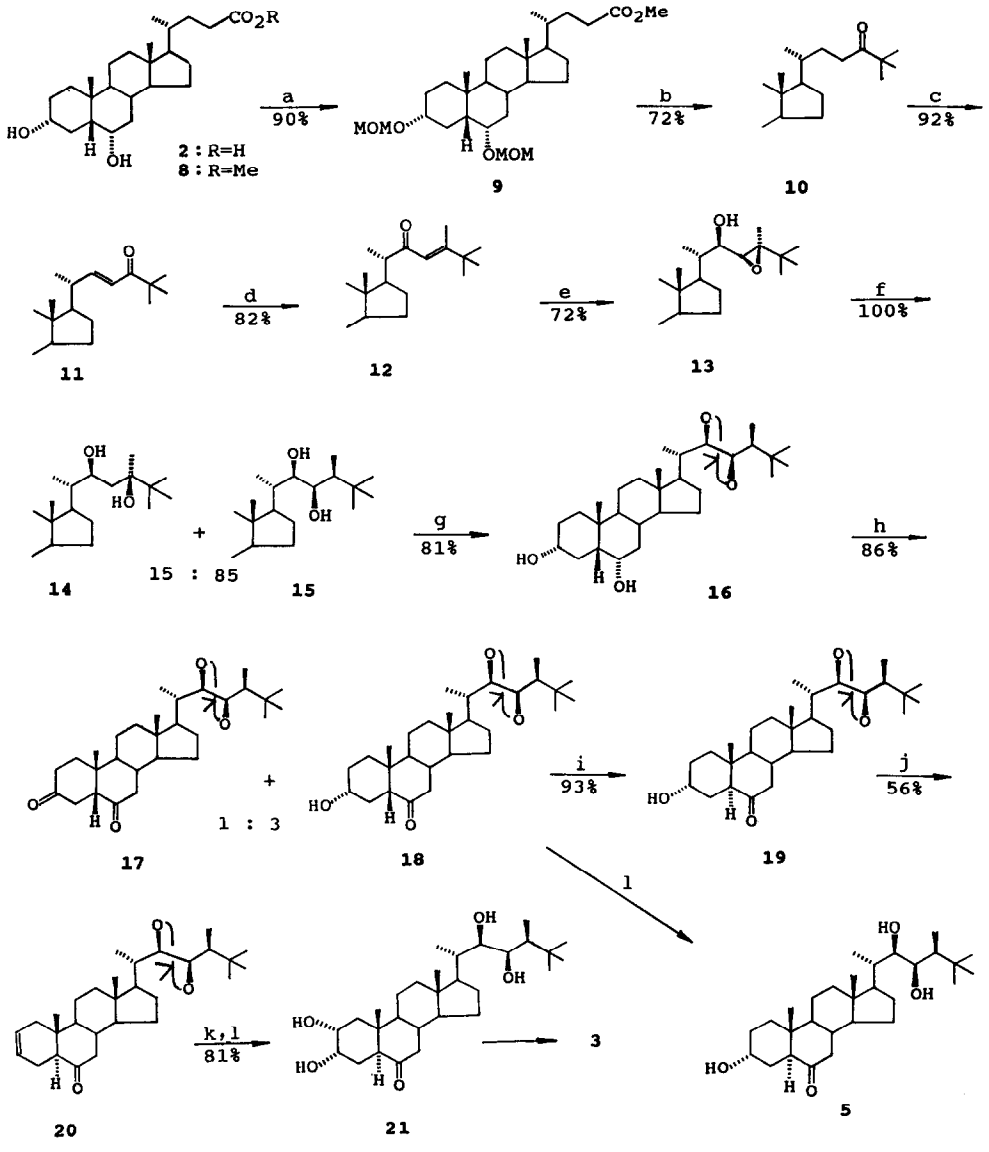
1. Synthesis of 25-methylbrassinolide (3) (Scheme 1)

Treatment of **9** , obtained from methyl hyodeoxycholate (**8**) (90% yield), with t-BuLi at -78°C afforded the ketone **10** in 72% yield. The Δ^{22}-ketone compound **11** was prepared by dehydrogenation of ketone compound **10** with PhSeBr-H_2O_2 system[8] in 92% yield. The oxidation of tertiary allylic alcohol generated by the 1,2-addition of methyllithium to Δ^{22}-ketone compound **11** with pyridinium chlorochromate (PCC), afforded the β-alkylative 1,3-carbonyl transposition product **12**[5]. The overall yield of these two-step reaction was 82%. The desired epoxide **13** was obtained by epoxidation with m-chloroperbenzoic acid (m-CPBA) in 72% yield.[6] Opening of the epoxide **13** with $LiBH_4$ in the presence of $Ti(OiPr)_4$ yielded quantitatively a mixture of the 1,2-diol **15** and 1,3-diol **14** in a ratio of 85:15 (HPLC)[9]. Removal of the 3α,6α-dihydroxy protecting group of **15** with pyridinium p-toluenesulfonate (PPTS)[10] followed by treatment with 2,2-dimethoxypropane, afforded the 22,23-acetonide **16** in 81% yield. 25-Methylcastasterone (**21**) mp. 261.5-262.5°C, $[\alpha]_D^{27}$ +13.04° (Lit.[6] mp. 251-253°C, $[\alpha]_D^{22}$ +14.3°), was prepared from **16** through the following sequence of reaction: **16→18→19→20→21** in 26% overall yield. Conversion of **21** into 25-methylbrassinolide (**3**) was known.[6] Conversion of **16** into a new 25-methyl-typhasterol (**5**) mp. 235-236°C, was achieved in 40% yield in two steps by oxidation with pyridinium dichromate (PDC) and the acid treatment for the epimerization of C_5. The overall yield for the construction of 25-methylbrassinosteroid sidechain (e.g **15**), starting from the methyl hyodeoxycholate (**8**), was ca. 30% in eight steps. This is one of the best method for constructing the sidechain of 25-methylbrassinosteroid.[6]

2. Synthesis of 26,27-Bisnorbrassinolide (**4**) (Scheme 2)

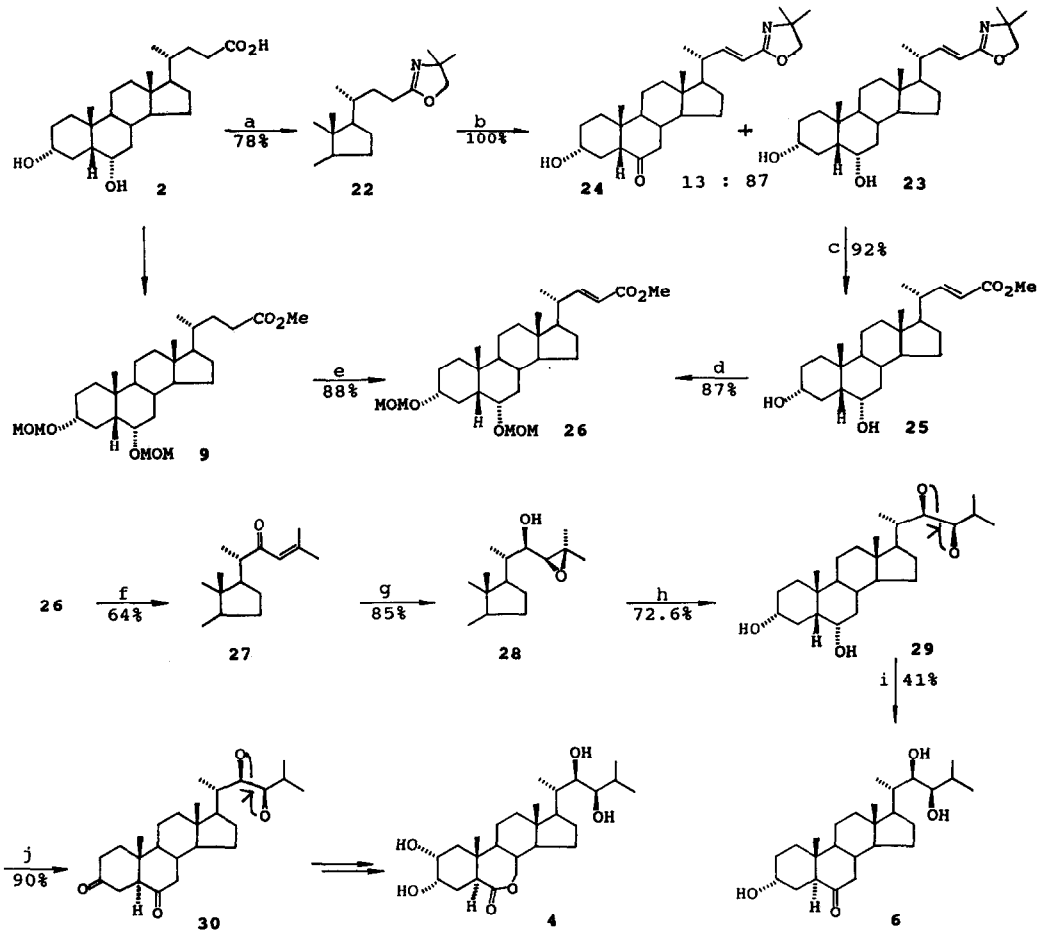
α,β-Unsaturated ester **26** used as a key intermediate, was prepared from hyodeoxycholic acid (**2**) via the oxazoline derivatives **22** and **23**. Thus, a mixture of **2** and 2-amino-2-methyl-1-propanol in the presence of a catalytic amount of boric acid, was heated in xylene to furnish **22** in 78% yield.[11] Dehydrogenation of **22** with $PhSeO_2H$ afforded a mixture of **23** (87%) and 24-[2-(4,4-dimethyl)-2-oxazolinyl]-5β-25,26,27-trinor-cholest-22-ene-3α-ol-6-one **24**(13%).[11] Δ^{22}-Methyl hyodeoxycholate **25**, which was obtained from the alcoholysis of **23** with 5% H_2SO_4/CH_3OH [12], was reacted with dimethoxymethane to provide the 3α,6α-dihydroxy protected unsaturated ester **26** in 80% yield in two steps. Compound **26** can also be obtained directly from dehydrogenation of **9** with PhSeBr-H_2O_2 system[8] in 88% yield. 22,23-Acetonide **29** was prepared from **26** in seven steps via **27** and **28** in the

Scheme 1



Reagents and conditions: a. $CH_2(OCH_3)_2$, P_2O_5, $CHCl_3$, r.t, 8h; b.t-BuLi, THF, -78°C, 15min; c. LDA, THF, -78°C; PhSeBr, -78--0°C, 1.5h; HOAc-H_2O, 30%H_2O_2, r.t,5h; d. 1. CH_3Li, THF, -78°C, 15min; 2. PCC, CH_2Cl_2, r.t, 36h; e. 1. DIBAL-H,THF,-78°C,30min; 2. m-CPBA,CH_2Cl_2,5°C,12h; f. $LiBH_4$, $Ti(OiPr)_4$, benzene, 10°C, 20h; g. 1. PPTS, t-BuOH, reflux, 5h; 2.$(MeO)_2CMe_2$, CH_2Cl_2, p-TsOH, r.t, 2h; h. PDC, CH_2Cl_2, r.t, 3h; i. CH_3ONa, CH_3OH, reflux, 30min; j. $CuSO_4$/silica gel, tetrachloroethylene, reflux, 6h; k. OsO_4, $K_3Fe(CN)_6$, t-BuOH:H_2O:THF(1:1:1), r.t, 24h; l. 2.5% HCl-CH_3OH, r.t, 36h.

Scheme 2



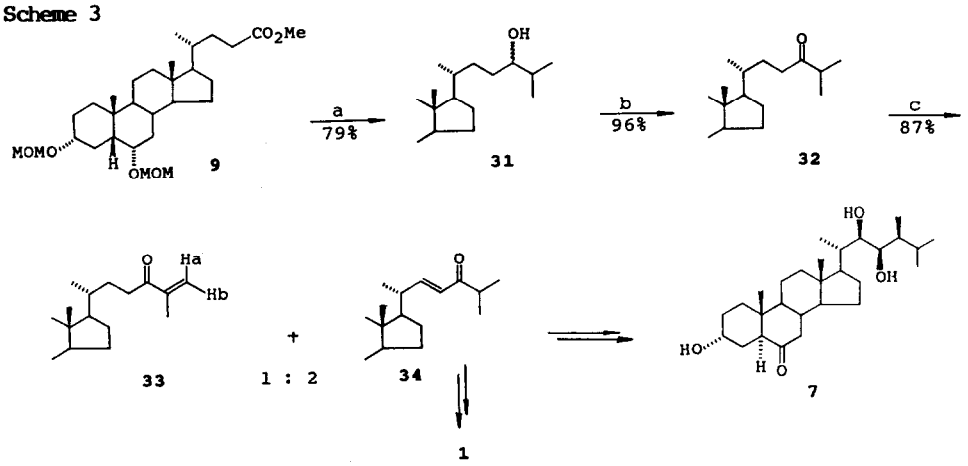
Reagents and conditions: a. 2-amino-2-methyl-1-propanol, H_3BO_3, xylene, reflux, 45h; b. $PhSeO_2H$, THF/pyridine, 60°C, 1h; c. 5% H_2SO_4-CH_3OH, reflux, 25-30h; d. $CH_2(OCH_3)_2$, P_2O_5, $CHCl_3$, r.t, 6h; e. LDA, THF, -78°C; PhSeBr, -78--0°C; HOAc-H_2O, 30%H_2O_2, r.t, 5h; f. 1. CH_3Li, THF, -78°C, 10min; 2. PCC, CH_2Cl_2, r.t, 18h; g. 1. DIBAL-H, THF, -78°C, 2h; 2. m-CPBA, CH_2Cl_2, 0-5°C, 8h; h. 1. $LiBH_4$, $Ti(OiPr)_4$, benzene, 5-10°C, 20h; 2. PPTS, t-BuOH, reflux, 3h; 3.$(MeO)_2CMe_2$, p-TsOH, CH_2Cl_2, r.t, 3h; i. 1. PDC, CH_2Cl_2, r.t, 2.5h; 2. 2.5% HCl-CH_3OH, r.t, 24h; j. 1. PDC, CH_2Cl_2, r.t, 24h; 2. p-TsOH, acetone, r.t, 24h.

same manner as **11–16** in 39.5% overall yield, involving β-alkylative 1,3-carbonyl transposition. Oxidation of **29** with PDC followed by 5-H epimerzation with p-toluenesulfonic acid (p-TsOH) yielded the known compound **30** mp. 164.5-165.5°C (Lit.[7d] 164-165°C) in 90% yield. The conversion of **30** into 26,27-bisnorbrassinolide (**4**) is known[7d]. Conversion of **29** to 26,27-bisnortyphasterol (**6**) mp. 225-226.5°C (Lit.[7e] 218-221°C) was accomplished in 41% yield by oxidation with PDC and the subsequent acid treatment.

The overall yield for the synthesis of the sidechain of 26,27-bisnorbrassinosteroid (e.g **29**), starting from methyl 3α,6α-di(methoxymethyl)hyodeoxycholate (**9**), was 35% in eight steps. To our knowledge, this is one of the best method for construction of these two compounds sidechain.[7]

3. Synthesis of brassinolide (**1**) (Scheme 3)

Scheme 3



Reagents and conditions: a. $iPrMgCl$-$LiBH_4$, THF, -25°C, 24h; b. PCC, CH_2Cl_2, r.t, 4h; c. LDA, THF, -78°C; PhSeBr, -78--0°C; HOAc-H_2O, 30% H_2O_2, r.t, 3h.

Treatment of **9** with $iPrMgCl$-$LiBH_4$ at -25°C[13] gave the epimeric mixture of alcohols **31** in 79% yield. Oxidation of **31** with PCC followed by dehydrogenation of the resulting ketone **32** with PhSeBr-H_2O_2 system[8] provided a ca. 2:1 mixture of Δ^{22}-compound **34** and Δ^{25}-compound **33** in 87% yield. The conversion of the pure **34** into brassinolide (**1**) and typhasterol (**7**) via β-alkylative 1,3-carbonyl transposition was known[2m].

In conclusion, the present method is simple, flexible, easy to perform and provide a high overall yield, in particular for the construction of the sidechain of 25-methyl- and 26,27-bisnorbrassinosteroids. In the mean time, the formal syntheses of brassinolide (**1**), 25-methylbrassinolide(**3**), 26,27-bisnorbrassinolide (**4**) were accomplished. 25-Methylcastasterone (**21**), 26,27-bisnortyphasterol (**6**) and the new 25-methyltyphasterol (**5**) were also synthesized.

EXPERIMENTAL

Melting points were determined on a Büchi 535 instrument and uncorrected. IR spectra were recorded on Shimadzu 440 spectrometer. ^{1}H-NMR spectra were obtained on a Varian XL-200(200MHz) and a JEOL SX-90 (90MHz) spectrometer, using $CDCl_3$ as solvent and TMS as an internal standard. Mass spectra were run on a JMS-01U spectrometer. High-resolution mass spectra were recorded with a Finnigan MAT 8430 spectrometer. The optical rotation was measured on Autpol III polarimeter. Elemental analyses were performed by the Analytical Department of this Institute. The work up as usual means that the extract was washed with 5% HCl (or saturated $NaHCO_3$), brine and dried ($MgSO_4$), then concentrated under reduced pressure. Flash column chromatography was performed on silica gel H (10-40 μ). Petroleum ether refers to the fraction boiling in the range 60-90°C.

Methyl 3α,6α-Di(methoxymethyl)hyodeoxycholate (9):

To a solution of 1.0 g (2.02 mmol) of methyl hyodeoxycholate (**8**) in dry chloroform (20 ml) were added freshly distilled dimethoxymethane (13 ml), and phosphorous pentoxide (3.0 g) with stirring. The reaction was then allowed to proceed at room temperature for 8 h. The mixture was poured into ice water, containing sodium carbonate, and the residue was washed with saturated aqueous sodium carbonate. The water solution was extracted with chloroform. After working up as usual the crude product was chromatographed on silica gel (petroleum ether /acetone 50:1) to afford 1.1 g of **9** as a colorless oily product in 90% yield, which was crystallized from CH_3OH-H_2O to give colorless needles. mp. 79.0-79.5°C; $[\alpha]_D^{22}$ +14.2° (c, 0.718, $CHCl_3$); IR (film): ν max: 1740 (C=O), 1145, 1100 (C-O) cm^{-1}; ^{1}H-NMR(90MHz): 0.60(3H, s, 18-H_3), 0.86(3H, s, 19-H_3), 0.87(3H, d, J=7Hz, 21-H_3), 2.12(2H, m, 23-H_2), 3.30(3H, s, OCH_3), 3.32(3H, s, OCH_3), 3.46(1H, m, 3β-H), 3.60(3H, s, $COOCH_3$), 3.82(1H, m, 6β-H), 4.57(2H, s, OCH_2O), 4.62(2H, s, OCH_2O); m/z 462(M^+-CH_3OH), 430(M^+-2CH_3OH), 370(M^+-2CH_3OCH_2OH); Found C, 70.34, H, 10.42, Calc. for $C_{29}H_{50}O_6$ C, 70.41, H, 10.19.

3α,6α-Dimethoxymethyloxy-5β-25-methyl-cholestan-24-one (10):

A 1.4M solution of tert-butyllithium (11.5 ml, 16.1 mmol) in pentane was added slowly dropwise via a syringe to a solution of 9 (6.5 g, 13.2 mmol) in dry THF (200 ml) under argon at -78°C with stirring. The mixture was continued to stir for 15 min and then quenched with aqueous NH_4Cl solution, and extracted with diethyl ether (3 x 100 ml). The extract was worked up as usual way to yield the crude product, which was purified by flash chromatography (petroleum ether /EtOAc 25:1) to give **10** (4.96 g,72.5%) as a colorless oil. $[\alpha]_D^{24}$ +13.94° (c, 1.15, $CHCl_3$); IR (film): ν max: 1710 (C=O), 1150, 1100, 1050 (C-O) cm^{-1}; ^{1}H-NMR(90MHz): 0.62(3H, s, 18-H_3), 0.88(3H, d, J=7Hz, 21-H_3), 0.90(3H, s, 19-H_3), 1.15(9H, s, 25-CH_3, 26-H_3, 27-H_3), 2.13(2H, t, J=8Hz, 23-H_2), 3.35(3H, s, OCH_3), 3.36(3H, s, OCH_3), 3.50(1H, m, 3β-H), 3.88(1H, m, 6β-H), 4.62(2H, s, OCH_2O), 4.67(2H, s,

OCH_2O); m/z: 521(M^++1), 520(M^+), 459(M^+-CH_3OCH_2O).

3α,6α-Dimethoxymethyloxy-5β-25-methyl-cholest-22(E)-en-24-one (11):

To a solution of diisopropylamine (1.46 ml, 10.4 mmol) and trace of 2,2 -dipyridine in dry THF (10 ml) was added dropwise a 1.95M solution of n-BuLi in hexane (5.4 ml, 10.5 mmol) at -20--30°C under nitrogen. This mixture was stirred at -20°C for 30 min. When this mixture was cooled to -78°C, a solution of **10** (3.1 g, 6.0 mmol) in THF (10 ml) was added dropwise. After stirring for an additional 1.5 h, a solution of PhSeBr (12 mmol) [prepared from PhSeSePh (2.3 g, 7.4 mmol) in THF (6 ml) and bromine (0.31 ml,6.0 mmol)] in THF (6 ml) was added rapidly, then the reaction temperature was raised to 0°C, water (5 ml) and acetic acid (2 ml) were added. After slow addition of 30% H_2O_2 (10 ml) at the temperature below 25°C, the reaction mixture was stirred for 5 h at 25°C and worked up as usual way. The crude product was purified by chromatography on silica gel (petroleum ether /EtOAc 25:1) to afford **11** (2.8 g, 92%) as a colorless oily product. $[\alpha]_D^{18}$ +14.55° (c, 1.45, $CHCl_3$); IR (film): ν max: 1690(C=O), 1620(C=C), 1150, 1100, 1050(C-O) cm^{-1}; ^{1}H-NMR(200MHz): 0.67(3H, s, 18-H_3), 0.91(3H, s, 19-H_3), 1.08(3H, d, J=6.6Hz, 21-H_3), 1.15(9H, s, 25-CH_3, 26-H_3, 27-H_3), 3.36(3H, s, OCH_3), 3.37(3H, s, OCH_3), 3.49(1H, m, 3α-H), 3.90(1H, m, 6β-H), 4.63(2H, s, OCH_2O), 4.66 and 4.71 (each 1H, each d, J=6.9Hz, OCH_2O), 6.40(1H, d, J=15.4Hz, 23-H), 6.77(1H, dd, J=9, 15.4Hz, 22-H); m/z: 518(M^+), 461 (M^+-CMe_3), 457(M^+-CH_3OCH_2O).

3α,6α-Dimethoxymethyloxy-5β-24,25-dimethyl-cholest-23(E)-en-22-one (12):

To a solution of the enone **11** (170 mg, 0.33 mmol) in THF (4 ml) at -78°C under nitrogen was added methyllithium (1.5M in diethyl ether, 0.4 ml, 0.6 mmol). The mixture was stirred for 15 min at -78°C and quenched with aq NH_4Cl solution and then extracted with diethyl ether (3x20 ml). The extract was worked up as usual to afford the crude product (170 mg) which was used directly for next step without further purification. To the crude product (50 mg) in dry CH_2Cl_2 (4 ml) was added PCC (170 mg), the mixture was stirred at room temperature for 36 h, and diluted with diethyl ether (6 ml). After working up as usual manner, the crude product was chromatographed (petroleum ether / EtOAc 20:1) to afford a colorless oil **12** (42 mg, 82%). $[\alpha]_D^{22}$ -7.92° (c, 1.39, $CHCl_3$); IR (film): ν max: 1680(C=O), 1610(C=C), 1150, 1100, 1050(C-O) cm^{-1}; ^{1}H-NMR(200MHz): 0.67 (3H, s, 18-H_3), 0.91(3H, s, 19-H_3), 1.09(3H, s, 21-H_3), 1.12(9H, s, 25-CH_3, 26-H_3, 27-H_3), 2.09(3H, d, J=1.0Hz, 24-CH_3), 2.47(1H, m, 20-H), 3.36(3H, s, OCH_3), 3.37(3H, s, OCH_3), 3.50(1H, m, 3β-H), 3.92(1H, m, 6β-H), 4.63(2H, s, OCH_2O), 4.67 and 4.73(each 1H, each d, OCH_2O), 6.11(1H, d, J=1.1Hz, 23-H); m/z: 533(M^++1), 532(M^+), 471(M^+-CH_3OCH_2O), 125($C_8H_{13}O$).

23,24-Epoxy-(22R)-22-hydroxy-3α,6α-dimethoxylmethyloxy-24,25-dimethyl-5β-cholestane(13):

To a solution of **12** (240 mg, 0.45 mmol) in THF (10 ml) at -78°C under nitrogen was

added DIBAL-H (1.0M in toluene, 1.3 ml, 1.3 mmol). The mixture was kept at -78°C for 30 min, and then quenched with methanol. After filtration, the solvent was removed under reduced pressure. The residue was treated with m-CPBA (80% purity, 320 mg) in dry CH_2Cl_2 (15 ml) and stirred at 5°C for 12 h. After usual work-up the crude product was chromatographed (petroleum ether /acetone 30:1) to give **13** (179 mg, 72%) as an amorphous solid. $[\alpha]_D^{22}$ -6.37° (c, 0.487, $CHCl_3$); IR (film): ν max: 3470(OH), 1150, 1100, 1050(C-O) cm^{-1}; ^{1}H-NMR(200MHz): 0.59(3H, s, 18-H_3), 0.84(3H, s, 19-H_3), 0.87(9H, s, 25-CH_3, 26-H_3, 27-H_3), 0.97(3H, d, J=6.3Hz, 21-H_3), 1.19(3H, s, 24-CH_3), 2.96(1H, d, J=6.0Hz, 23-H), 3.29 (3H, s, OCH_3), 3.30(3H, s, OCH_3), 3.45(1H, m, 3β-H), 3.53(1H, d, J=6.0Hz, 22-H), 3.84 (1H, m, 6β-H), 4.56(2H, s, OCH_2O), 4.61 and 4.64 (each 1H, each d, OCH_2O); m/z: 551(M^+ +1), 549(M^+-1), 493(M^+-CMe_3); Found C, 72.12, H, 11.01, Calc. for $C_{33}H_{58}O_6$ C, 71.96, H, 10.61.

(22R,23R,24S)-22,23-Dihydroxy-3α,6α-dimethoxymethyloxy-5β-24,25-dimethyl-cholestane (15) and (22R,24S)-22,24-dihydroxy-3α,6α-dimethoxylmethyloxy-5β-24,25-dimethyl-cholestane (14):

Titanium tetraisopropoxide (0.35 ml, 0.12mmol) was added to a solution of epoxy alcohol **13** (45 mg, 0.08 mmol) in dry benzene (2 ml) under nitrogen with stirring at room temperature. After stirring for 10min, the mixture was cooled to 10°C, and $LiBH_4$ (80 mg, 3.68 mmol) was then added. The mixture was continued to stir for 20 h, and then diluted with diethyl ether (4 ml) and 5% sulfuric acid with vigorously stirring till two clear layers were separated. After working up as usual, a mixture of 1,2-diol **15** and 1,3-diol **14** was obtained quantitatively in a ratio of 85:15 (HPLC analysis). Compound **14** and **15** can be readly separated by column chromatography (petroleum ether/ acetone 20:1).

1,2-Diol **15** : IR (KCl): ν max: 3350(OH), 1140, 1100, 1040(C-O) cm^{-1}; ^{1}H-NMR(200MHz): 0.59(3H, s, 18-H_3), 0.78(3H, d, J=7.2Hz, 21-H_3), 0.79(3H, d, J=6.8Hz, 24-CH_3), 0.84(3H, s, 19-H_3), 0.88(9H, s, 25-CH_3, 26-H_3, 27-H_3), 3.29(3H, s, OCH_3), 3.30(3H, s, OCH_3), 3.41(1H, d, J=8.8Hz, 23-H), 3.46(1H, m, 3β-H), 3.74(1H, d, J=8.8Hz, 22-H), 3.86(1H, m, 6β-H), 4.56(2H, s, OCH_2O), 4.59 and 4.63(each 1H, each d, J=6.8Hz, OCH_2O); m/z: 552(M^+), 551(M^+-1), 520(M^+-CH_3OH), 495(M^+-CMe_3; Found C, 67.60, H, 10.76, Calc. for $C_{33}H_{60}O_6 \cdot 2H_2O$ C, 67.31, H, 10.95.

1,3-Diol **14** : ^{1}H-NMR(200MHz): 3.30(3H, s, OCH_3), 3.31(3H, s, OCH_3), 3.48(1H, m, 3β-H), 3.86(1H, m, 6β-H), 4.10(1H, m, 22-H), 4.57(2H, s, OCH_2O), 4.61 and 4.65(each 1H, each d, J=6.9Hz, OCH_2O).

3α,6α-Dihydroxy-5β-(22R,23R,24S)-22,23-isopropylidenedioxy-24,25-dimethyl-cholestane (16):

A mixture of **15** (100 mg, 0.18 mmol), PPTS(200 mg, 1.04 mmol) and t-BuOH (5 ml) was heated under reflux with stirring for 5 h. After working up as usual manner, the residue, dissolved in dry CH_2Cl_2 (2 ml), was treated with 2,2-dimethoxypropane (1 ml) and

p-TsOH (10 mg) at room temperature for 2 h. After working up as usual, the residue was chromatographed on silica gel (petroleum ether/ EtOAc 3:2) to afford **16** (74 mg, 81%). mp. 268-270°C (prisms from THF-acetone); $[\alpha]_D^{27}$ +31.2° (c, 0.52, $CHCl_3$); IR (KCl): ν max: 3350(OH), 1220, 1160, 1120, 1020 cm^{-1}; ^{1}H-NMR(200MHz): 0.66(3H, s, 18-H_3), 0.87(3H, d, J=6.9Hz, 21-H_3), 0.89(3H, s, 19-H_3), 0.91(9H, s, 25-CH_3, 26-H_3, 27-H_3), 0.97(3H, d, J=6.5Hz, 24-CH_3), 1.34(6H, s, =CMe_2), 3.64(1H, m, 3β-H), 3.67(1H, d, J=9.3Hz, 23-H), 3.93 (1H, d, J=9.3Hz, 22-H), 4.08(1H, m, 6β-H); m/z: 504(M^+), 489(M^+-CH_3), 419(M^+-C_6H_{13}), 185($C_{11}H_{21}O_2$); Found C, 75.98, H, 11.44, Calc. for $C_{32}H_{56}O_4$ C, 76.14, H, 11.18.

(22R,23R,24S)-22,23-Isopropylidenedioxy-24,25-dimethyl-5β-cholestane-3α-ol-6-one (18) and (22R,23R,24S)-22,23-isopropylidenedioxy-24,25-dimethyl-5β-cholestane-3,6-dione (17):

A solution of **16** (200 mg, 0.40 mmol) in CH_2Cl_2 was treated with PDC (240 mg) at room temperature for 3 h. Working up in the usual way gave a mixture which was separated by chromatography on silica gel to provide 3,6-dione **17** (47 mg, 24%) and 3α-ol-6-one **18** (124 mg, 62%).

17 : mp. 201-202°C; $[\alpha]_D^{26}$ -5.2° (c, 0.998, $CHCl_3$); IR (nujol): ν max: 1710(C=O), 1230, 1020(C-O) cm^{-1}; ^{1}H-NMR(200MHz): 3.68(1H, d, J=9.5Hz, 23-H), 3.95(1H, d, J=9.5Hz, 22-H); m/z: 501(M^++1), 485(M^+-CH_3), 443(M^+-CMe_3), 185($C_{11}H_{21}O_2$); Found C, 76.24, H, 10.70, Calc. for $C_{32}H_{52}O_4.1/4H_2O$ C, 76.07, H, 10.47.

18 : mp. 282.5-284.5°C; $[\alpha]_D^{25}$ -20.20°(c, 1.49, $CHCl_3$); IR (KCl): max: 3450(OH), 1710 (C=O) cm^{-1}; ^{1}H-NMR(200MHz): 0.67(3H, s, 18-H_3), 0.85(3H, s, 19-H_3), 0.88(3H, d, J=6.9Hz, 21-H_3), 0.91(9H, s, 25-CH_3, 26-H_3, 27-H_3), 0.98(3H, d, J=6.6Hz, 24-CH_3), 1.35(6H, s, =CMe_2), 3.66(1H, d, J=9.2Hz, 23-H), 3.67(1H, m, 3β-H), 3.93(1H, d, J=9.2Hz, 22-H); m/z: 503(M^++1), 487(M^+-CH_3), 417(M^+-C_6H_{13}), 185($C_{11}H_{21}O_2$); Found C, 74.86, H, 10.77, Calc. for $C_{32}H_{54}O_4.1/2H_2O$ C, 75.10, H, 10.83.

(22R,23R,24S)-22,23-Isopropyldenedioxy-24,25-dimethyl-5α-cholestane-3α-ol-6-one (19):

A solution of **18** (60 mg, 0.12 mmol) and 50% sodium methoxide (50 mg) in methanol (3 ml) was heated under reflux for 30 min. CH_2Cl_2 (30 ml) was added to the mixture, and then worked up as usual. Purification of this crude product on silica gel (petroleum ether/ EtOAc 2:1) provided **19** (56 mg, 93%) as cololess needles. mp.260-261.2°C ($CHCl_3$-EtOAc); $[\alpha]_D^{25}$ +22.50° (c, 0.396, $CHCl_3$); IR (KBr): ν max: 3450(OH), 1710(C=O) cm^{-1}; ^{1}H-NMR(200MHz): 0.68(3H, s, 18-H_3), 0.73(3H, s, 19-H_3), 0.87(3H, d, J=7.1Hz, 21-H_3), 0.91(9H, s, 25-CH_3, 26-H_3, 27-H_3), 0.99(3H, d, J=6.5Hz, 24-CH_3), 1.34(6H, s, =CMe_2), 2.30(1H, dd, J=12.7, 4.2Hz, 7β-H), 2.73(1H, t, J=8Hz, 5α-H), 3.66(1H, d, J=9.2Hz, 23-H), 3.93(1H, d, J=9.2Hz, 22-H), 4.17(1H, $W_{\frac{1}{2}}$=7Hz, 3β-H); m/z: 503(M^++1), 487(M^+-CH_3), 417(M^+-C_6H_{13}), 185($C_{11}H_{21}O_2$); Found C, 76.44, H, 10.94, Calc. for $C_{32}H_{54}O_4$ C, 76.45, H, 10.83.

(22R,23R,24S)-22,23-Isopropylidenedioxy-24,25-dimethyl-5α-cholestane-2-en-6-one (20):

A mixture of **19** (105 mg, 0.21 mmol), the catalyst [14] (600 mg, 0.66 mmol $CuSO_4$) and

tetrachloroethylene was heated under reflux with stirring for 6 h, and then the catalyst was filtered off. Concentration under reduced pressure provided a crude product, which was purified by column chromatography (petroleum ether/ EtOAc 30:1) to afford Δ^2-6-one **20** (57 mg, 56%) as colorless needles. mp. 269-270°C; $[\alpha]_D^{19}$ +46.15°(c, 0.26, $CHCl_3$); IR (KBr): νmax: 1710(C=O), 1660(C=C), 670(=⌒H) cm^{-1}; ^{1}H-NMR(200MHz): 0.69(3H, s, 18-H_3), 0.71(3H, s, 19-H_3), 0.87(3H, d, J=6.8Hz, 21-H_3), 0.91(9H, s, 25-CH_3, 26-H_3, 27-H_3), 0.99 (3H, d, J=6.5Hz, 24-CH_3), 1.34(6H, s, =CMe_2), 3.67(1H, d, J=9.3Hz, 23-H), 3.94(1H, d, J= 9.3Hz, 22-H), 5.62(2H, m, 2-H,3-H); m/z: 485(M^++1), 484(M^+), 469(M^+-CH_3), 185($C_{11}H_{21}O_2$); Found C, 79.52, H, 10.93, Calc. for $C_{32}H_{52}O_3$ C, 79.29, H, 10.81.

25-Methylcastasterone (21):

The OsO_4 solution (0.05 M in t-BuOH, 0.1 ml) was added to a mixture of **20** (20 mg, 0.04 mmol), $K_3Fe(CN)_6$ (198 mg, 0.6 mmol) and K_2CO_3 (83 mg, 0.6 mmol) in a mixed solvent (t-BuOH /THF /H_2O=1:1:1, 3 ml)[15]. The reaction mixture was stirred for 24 h at room temperature, Na_2SO_3 (100 mg) was added, and the resulting mixture was stirred for 1 h. After concentration under reduced pressure, the residue was diluted with $CHCl_3$ (20 ml). After working up as usual, the residue dissolved in 2.5% HCl-CH_3OH(3 ml) was allowed to stand for 36 h for removing the side chain protected group and then worked up as usual. Purification by column chromatography on silica gel ($CHCl_3$/ CH_3OH 20:1) afforded the title compound **21** (16 mg, 81%) as colorless needles. mp.261.5-262.5°C(lit.[6] 251-253°C); $[\alpha]_D^{27}$ +13.04° (c, 0.30, CH_3OH) [Lit.[6] $[\alpha]_D^{22}$ +14.3° (c, 0.11, CH_3OH)]; IR (KBr): ν max: 3400(OH), 1710(C=O)cm^{-1}; ^{1}H-NMR(200MHz, 45.7°C): 0.69(3H, s, 18-H_3), 0.76(3H, s, 19-H_3), 0.85(3H, d, J=7.1Hz, 24-CH_3), 0.92(3H, J=6.2Hz, 21-H_3), 0.96(9H, s, 25-CH_3, 26-H_3, 27-H_3), 2.30(1H, dd, J=12.7, 4.1Hz, 7β-H), 2.68(1H, dd, J=12.5, 3.5Hz, 5α-H), 3.48(1H, d, J=9.0Hz, 23-H), 3.78(1H, m, 2-H), 3.81(1H, J=9.0Hz, 22-H), 4.03(1H, $W_{\frac{1}{2}}$ =8.0Hz, 3β-H); m/z: 461(M^+-OH), 443(M^+-OH-H_2O), 393(M^+-C_6H_{13}).

25-Methyltyphasterol (5):

A solution of **16** (60 mg, 0.12 mmol) in CH_2Cl_2 (10 ml) was treated with PDC (90 mg) at room temperature for 3 h. The mixture was diluted with dry diethyl ether (10 ml) and the solid was filtered. After removal of solvent, the residue dissolved in 2.5% HCl-CH_3OH (3 ml) was allowed to stand overnight and then worked up as usual. On purification by chromatography (petroleum ether/ EtOAc 1:1) **5** (22 mg, 40%) as a colorless needle was obtained. mp.235-236°C; IR (KBr): ν max: 3400(OH), 1710(C=O)cm^{-1}; ^{1}H-NMR(200MHz): 0.69 (3H, s, 18-H_3), 0.74(3H, s, 19-H_3), 0.85(3H, d, J=7.0Hz, 24-CH_3), 0.92(3H, d, J=6.2Hz, 21-H_3), 0.95(9H, s, 25-CH_3, 26-H_3, 27-H_3), 2.31(1H, dd, J=12.8, 4.3Hz, 7β-H), 2.73(1H, t, J=8.3Hz, 5α-H), 3.48(1H, d, J=8.6Hz, 23-H), 3.82(1H, d, J=8.6Hz, 22-H), 4.17(1H, $W_{\frac{1}{2}}$ =7.5Hz, 3-H); m/z: 463(M^++1), 445(M^+-OH), 427(M^++1-2H_2O); HRMS m/z: 349.2738 (M^++2-$C_7H_{15}O$, required 349.2743 $C_{22}H_{37}O_3$), 348.2690(M^++1-$C_7H_{15}O$, required 348.2664 $C_{22}H_{36}O_3$), 347.2575 (M^+-$C_7H_{15}O$, required 347.2586 $C_{22}H_{35}O_3$), 329.2449(M^+-$C_7H_{15}O$-H_2O, required

329.2480 $C_{22}H_{33}O_2$).

Methyl 3α,6α-Di(methoxymethyl)-22,23-dehydrohyodeoxycholate (26):

The dehydrogenation procedure was carried out in the same way as **11** : LDA (10.7 mmol), **9** (2.8 g, 5.7 mmol) and PhSeBr (12.0 mmol) was used. 88% of **26** (2.46 g) was obtained as a colorless oil, which was crystallized from hexane to afford a fine colorless needle. mp. 74-74.5°C; $[\alpha]_D^{14}$ +7.05° (c, 1.305, $CHCl_3$); IR (film): ν max: 1710(C=O), 1640(C=C), 1130, 1090, 1040(C-O) cm^{-1}; ^{1}H-NMR(90MHz): 0.65 (3H, s, 18-H_3), 0.84(3H, s, 19-H_3), 1.05(3H, d, J=7Hz, 21-H_3), 3.32(3H, s, OCH_3), 3.34(3H, s, OCH_3), 3.46(1H, m, 3β-H), 3.68(3H, s, $COOCH_3$), 3.90(1H, m, 6β-H), 4.58(2H, s, OCH_2O), 4.65(2H, s, OCH_2O), 5.68 (1H, d, J=16Hz, 23-H), 6.78(1H, dd, J=16, 9Hz, 22-H); m/z: 492(M^+), 369(M^+-CH_3OCH_2O-CH_3OCH_2OH), 368(M^+-2CH_3OCH_2OH); Found C, 70.68, H, 9.83, Calc. for $C_{29}H_{48}O_6$ C, 70.70, H, 9.82.

24-[2-(4,4-Dimethyl)-2-oxazolinyl]-5β-25,26,27-trinor-cholestane-3α,6α-diol (22):

A mixture of hyodeoxycholic acid (**2**) (14.0 g, 35.7 mmol), 2-amino-2-methyl-1-propanol (6.0 g, 67 mmol), boric acid (1.15 g, 18.6 mmol) and xylene(270 ml) was heated in reflux with azeotropic removal of water for 45 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (petroleum ether/ EtOAc 1:1) to afford 12.45 g (78.3%) of oxazoline **22** as a colorless needle. mp. 164-165°C (lit.[16] 165 -166°C); IR (nujol): ν max: 3400(OH), 1640(C=N), 1120 cm-1; 1H-NMR(90MHZ): 0.62(3H, s, 18-H_3), 0.84(3H, s, 19-H_3), 0.92(3H, d, J=7Hz, 21-H_3), 1.25(6H, s, =CMe_2), 2.24(2H, m, 23-H), 3.58(1H, m, 3β-H), 3.98(2H, s, OCH_2-); m/z: 446(M^++1), 445(M^+).

24-[2-(4,4-Dimethyl)-2-oxazolinyl]-5β-25,26,27-trinor-cholest-22-ene-3α,6α-diol (23) and 24-[2-(4,4-dimethyl)-2-oxazolinyl]-5β-25,26,27-trinor-cholest-22-ene-3α-ol-6-one (24):

A solution of the oxazoline 22 (133 mg, 0.3 mmol) in dry THF (7 ml) and pyridine (1.3 ml) was heated to 60°C. Benzeneseleninic acid (240 mg, 1.3 mmol) was added and the reaction mixture was stirred at 60°C for 1 h. After cooling to room temperature, 30% H_2O_2 (2.5 ml) was added with vigorous stirring for 30 min and 5% K_2CO_3 (3.8 ml) was added. The mixture was extracted with CH_2Cl_2. The extract was worked up as usual. Purification by chromatography on silica gel gave **24** (17 mg, 13%) and **23** (115 mg, 87%).

23 : mp. 205-205.5°C (needles from EtOAc); $[\alpha]_D^{30}$ -4.73° (c, 0.46, CH_3OH); IR (KCl): ν max: 3350(OH), 1660(C=N), 1600(C=C), 1040 cm^{-1}; ^{1}H-NMR(90MHz): 0.65(3H, s, 18-H_3), 0.89(3H, s, 19-H_3), 1.06(3H, d, J=7Hz, 21-H_3), 1.32(6H, s, =CMe_2), 3.56(1H, m , 3β-H), 3.97(2H, s, OCH_2-), 4.02(1H, m, 6β-H), 5.86(1H, d, J=16.2Hz, 23-H), 6.48(1H, dd, J=16.2, 9Hz, 22-H); m/z: 444(M^++1), 443(M^+); Found C, 75.18, H, 10.43, N, 3.02, Calc. for $C_{28}H_{45}O_3N.1/4H_2O$ C, 75.04, H, 10.23, N, 3.13.

24 : mp. 211.5-212.3°C (scale from acetone); $[\alpha]_D^{30}$ -39.40°(c, 0.46, CH_3OH); IR (KCl): ν max: 3350(OH), 1700(C=O), 1660(C=N), 1605(C=C) cm^{-1}; ^{1}H-NMR(90MHz): 0.65(3H,s, 18-H_3),

0.81(3H, s, 19-H_3), 1.05(3H, d, J=7Hz, 21-H_3), 1.28(6H, s, =CMe_2), 2.14(3H, m, 5-H, 7-H_2), 3.58(1H, m, 3β-H), 3.92(2H, s, OCH_2-), 5.83(1H, d, J=16Hz, 23-H), 6.43(dd, J=16, 9Hz, 22-H); m/z: 442(M^++1), 441(M^+); Found C, 75.43, H, 9.86, N, 3.01, Calc. for $C_{28}H_{43}O_3N.1/4H_2O$ C, 75.38, H, 9.83, N, 3.14.

Methyl 22,23-Didehydrohyodeoxycholate (25):

Compound **23** (600 mg, 1.36 mmol) in 10ml of 5% H_2SO_4-CH_3OH was heated under reflux for 25-30 h. After cooling, the solvent was removed under reduced pressure. The residue was extracted with CH_2Cl_2 (50 ml). The extract was worked up as usual to give the crude product, which was purified by flash chromatography on silica gel (petroleum ether /acetone 5:1) to give pure **25** (501 mg, 92%) as a colorless solid. mp. 74-78°C (benzene); $[\alpha]_D^{24}$ -2.76° (c, 1.811, $CHCl_3$); IR (film): ν max: 3350(OH), 1720(C=O), 1650(C=C) cm^{-1}; ^{1}H-NMR (200MHz): 0.69(3H, s, 18-H_3), 0.93(3H, s, 19-H_3), 1.09(3H, d, J=6.6Hz, 21-H_3), 3.68(1H, m, 3β-H), 3.73(3H, s, OCH_3), 4.10(1H, m, 6β-H), 5.75(1H, d, J=16Hz, 23-H), 6.84(1H, dd, J=16, 9Hz, 22-H); m/z: 405(M^++1), 404(M^+), 386(M^+-H_2O), 368(M^+-$2H_2O$); Found C, 73.98, H, 10.42, Calc. for $C_{25}H_{40}O_4$ C, 74.22, H, 9.97.

Methyl 3α,6α-Di(methoxymethyl)-22,23-dehydrohyodeoxycholate (26):

3α,6α-Diol **25** (150 mg, 0.37 mmol) was protected with dimethoxymethane (3 ml, 34 mmol) as described for that of 3α,6α-diol **8** to give α,β-unsaturated ester **26** (159 mg, 87%). The spectral data were identical with those described as above.

3α,6α-Dimethoxymethyloxy-5β-26,27-bisnor-cholest-23(E)-en-22-one (27):

1,3-Carbonyl transposition of **27** was carried out as described for preparation of **12** by using of **26** (2.0g, 4 mmol), CH_3Li (1.0 M in diethyl ether, 10 ml) and PCC (3.4 g) in CH_2Cl_2. Compound **27** (1.28 g, 64%) was obtained as a colorless oil. $[\alpha]_D^{25}$ -18.37° (c, 0.566, $CHCl_3$); IR (film): ν max: 1680(C=O), 1620(C=C), 1150, 1100, 1040(C-O) cm^{-1}; ^{1}H-NMR (200MHz): 0.59(3H, s, 18-H_3), 0.84(3H, s, 19-H_3), 1.03(3H, d, J=6.9Hz, 21-H_3), 1.83(3H, d, J=0.7Hz, 24-CH_3), 2.05(3H, s, 24-CH_3), 2.38(1H, m, 20-H), 3.28(3H, s, OCH_3), 3.30(3H, s, OCH_3), 3.47(1H, m, 3β-H), 3.86(1H, m, 6β-H), 4.46(2H, s, OCH_2O), 4.60 and 4.64(each 1H, each d, J=7Hz, OCH_2O), 6.00(1H, s, 23-H); m/z: 491(M^++1), 490(M^+), 475(M^+-CH_3).

(22R,23S)-23,24-Epoxy-3α,6α-dimethoxymethyloxy-24-methyl-26,27-bisnor-5β-cholestane(28):

The reaction was carried out in the same way as **13** : **27** (780 mg, 1.6 mmol) was treated with DIBAL-H (1.0M in toluene, 6 ml) followed by epoxidation of the resulting allylic alcohol compound with mCPBA (80% purity, 960 mg) to afford **28** (690 mg, 85%) as a colorless solid. mp. 109-110°C (hexane); $[\alpha]_D^{24}$ -11.46° (c, 0.637, $CHCl_3$); IR (film): ν max: 3500(OH), 1160, 1110, 1050(C-O) cm^{-1}; ^{1}H-NMR(200MHz): 0.59(3H, s, 18-H_3), 0.84 (3H, s, 19-H_3), 0.96(3H, d, J=6.1Hz, 21-H_3), 1.26(3H, s, 24-CH_3), 1.27(3H, s, 25-H_3), 2.76(1H, d, J=6.0Hz, 23-H), 3.29(3H, OCH_3), 3.30(3H, s, OCH_3), 3.45(1H, m, 3β-H), 3.52

(1H, d, J=6.0Hz, 22-H), 3.84(1H, m, 6β-H), 4.56(2H, s, OCH_2O), 4.59 and 4.64 (each 1H, each d, J=6.9Hz, OCH_2O); m/z: 509(M^++1), 491(M^+-OH), 476(M^+-CH_3OH), 447(M^+-CH_3OCH_2O); Found C, 70.86, H, 10.67, Calc. for $C_{30}H_{52}O_6$ C, 70.83, H, 10.30.

(22R,23R)-22,23-Isopropylidenedioxy-24-methyl-5β-26,27-bisnorcholestane-3,6-diol (29):

$Ti(OiPr)_4$ (0.45 ml, 0.15 mmol) was added to a solution of the epoxy alcohol **28** (50 mg, 0.1 mmol) in dry benzene (2 ml) under nitrogen with stirring at room temperature for 10 min then $LiBH_4$ (80 mg, 3.68 mmol) was added. The mixture was stirred for 20 h at 5-10°C and then treated in the same manner as **14** . The mixture of the resulting crude product and PPTS (100 mg) was heated in refluxing t-BuOH (3 ml) for 3 h. After cooling, CH_2Cl_2 (50 ml) was added to the mixture and the organic layer was washed with water and brine and dried ($MgSO_4$) and concentrated to dryness under reduced pressure to give the residue which was dissolved in dry CH_2Cl_2(1 ml) and treated with 2,2-dimethoxypropane (1ml) and p-TsOH(10 mg) at room temperature to stand for 3 h. Usual workup followed by chromatography (petroleum ether/ EtOAc 2:1) gave **29** (33 mg, 72.6%) as a colorless needle. mp. 202-203°C (EtOAc); $[\alpha]_D^{22}$ +9.85°(c, 0.325, $CHCl_3$); IR (KBr): ν max: 3400(OH), 1240, 1040 cm^{-1}; ^{1}H-NMR(200MHz): 0.64(3H, s, 18-H_3), 0.91(3H, s, 19-H_3), 0.92(3H, d, J=6.8Hz, 25-H_3), 0.96(3H, d, J=5.4Hz, 21-H_3), 0.99(3H, d, J=6.6Hz, 24-CH_3), 1.35 and 1.39 (2x3H, 2s, =CMe_2), 3.44(1H, dd, J=7.7, 7.7Hz, 23-H), 3.62(1H, m, 3β-H), 3.95(1H, d, J=7.7Hz, 22-H), 4.06(1H, m, 6β-H); m/z: 448(M^++1-CH_3), 447(M^+-CH_3), 419(M^+-C_3H_7), 143($C_8H_{15}O_2$); Found C, 74.84, H, 10.95, Calc for $C_{29}H_{50}O_4.1/8H_2O$ C, 74.91, H, 10.89.

(22R,23R)-22,23-Isopropyldenedioxy-24-methyl-5α-cholestane-3,6-dione (30):

A solution of **29** (50 mg) in CH_2Cl_2 (10 ml) was treated with PDC (120 mg) at room temperature for 24 h. The mixture was diluted with dry diethyl ether (10 ml) and the solid was filtered. After removal of solvent, the residue dissolved in acetone (2 ml) was treated with p-TsOH (10 mg) to stand for 24 h at room temperature. Removal of solvent followed by chromatography afforded **30** (45 mg, 90%) as a colorless needle. mp. 164.5-165.5°C (Lit.[7c] 164-165°C); IR(KBr): ν max: 1710(C=O)cm^{-1}; ^{1}H-NMR(200MHz): 0.69 (3H, s, 18-H_3), 0.91(3H, d, J=6.9Hz, 21-H_3), 0.96(3H, s, 19-H_3), 0.98(3H, d, J=6.7Hz, 25-H_3), 0.99(3H, d, J=6.7Hz, 24-CH_3), 1.35 and 1.39 (2x3H, 2s, =CMe_2), 3.45(1H, dd, J= 7.3,7.3Hz, 23-H), 3.91(1H, d, J=7.3Hz, 22-H); m/z: 459(M^++1), 444(M^++1-CH_3), 443(M+-CH_3), 143($C_8H_{15}O_2$); Found C, 75.14, H, 10.07, Calc. for $C_{29}H_{46}O_4.1/4H_2O$ C, 75.20, H, 10.12.

26,27-Bisnortyphasterol (6):

Oxidation reaction was performed as described for **5** : **29** (80 mg) was treated with PDC(100 mg) in CH_2Cl_2 (10 ml), followed by treatment with 2.5% HCl-CH_3OH to afford the title compound **6** (30 mg, 41%) as a colorless needle. mp. 225.5-226.5°C (Lit.[7e] 218-221°C); IR(KBr): ν max: 3400(OH), 1710(C=O) cm^{-1}; ^{1}H-NMR(200MHz): 0.68(3H, s, 18-H_3), 0.73(3H, s, 19-H_3), 0.86(3H, d, J=6.6Hz, 21-H_3), 0.93(3H, d, J=6.0Hz, 25-H_3), 1.02(3H,

d, J=6.7Hz, 24-CH_3), 2.31(1H, dd, J=12.7, 3.9Hz, 7β-H), 2.73(1H, t, J=7.8Hz, 5α-H), 3.44 (1H, dd, J=7.8, 2.5Hz, 23-H), 3.57(1H, d, J=7.8Hz, 22-H), 4.17(1H, $W_{\frac{1}{2}}$=7Hz, 3-H); m/z: 421(M^++1), 420(M^+), 403(M^+-OH), 377(M^+-C_3H_7); Found C, 72.39, H, 10.61, Calc. for $C_{26}H_{44}O_4.1/2H_2O$ C, 72.68, H, 10.56.

3α,6α-Dimethoxymethyloxy-5β-cholestane-24-ol (31):

A solution of iPrMgCl (2.0 M in THF, 5.8 ml) was added to $LiBH_4$ (43 mg, 2.0 mmol) in THF (5 ml) under argon. The stirred mixture was cooled to -23°C and compound **9** (480 mg, 0.97 mmol) in THF (5 ml) added dropwise via a syringe, then THF (5 ml) was added to the mixture. After stirring for 10 min, the reaction flask was placed in a freezer (-25°C) for 24 h. The reaction was quenched by careful addition of 5% HCl. The mixture was extracted with diethyl ether. The extract was worked up as usual followed by purification on silica gel column (petroleum ether/ acetone 40:1) to afford **31** (390 mg, 79%) as a colorless oil. IR(film): ν max: 3450(OH), 1150, 1100, 1050 cm^{-1}; ^{1}H-NMR(90MHz): 0.62 (3H, s, 18-H_3), 0.88(3H, s, 19-H_3), 0.90(6H, d, J=7Hz, 26-H_3, 27-H_3), 3.30(3H, s, OCH_3), 3.34(3H, s, OCH_3), 3.18-3.60(2H, m, 3β-H, 24-H), 3.84(1H, m, 6β-H), 4.59(2H, s, OCH_2O), 4.64(2H, s, OCH_2O); m/z: 508(M^+), 476(M^+-CH_3OH), 446(M^+-CH_3OCH_2OH), 444(M^+-$2CH_3OH$).

3α,6α-Dimethoxymethyloxy-5β-cholestane-24-one (32):

Compound **31** (150 mg, 0.29 mmol) in CH_2Cl_2 (5 ml) was added to a stirred suspension of PCC (152 mg) and NaOAc (58 mg) in dry CH_2Cl_2 (5 ml). The mixture was stirred at room temperature for 4 h and diluted with diethyl ether (20 ml) and then the solid was filtered. Removal of solvent under reduced pressure to dryness gave the ketone **32** (146 mg, 96%) as a colorless oil. $[\alpha]_D^{24}$ +12.8°(c, 0.66, $CHCl_3$); IR(film): ν max: 1725 (C=O), 1150, 1110, 1050 cm^{-1}; ^{1}H-NMR(200MHz): 0.64(3H, s, 18-H_3), 0.91(3H, d, J=6Hz, 21-H_3), 0.93(3H, s, 19-H_3), 1.10(6H, d, J=6.9Hz, 26-H_3, 27-H_3), 2.40(2H, m, 23-H_2), 2.61 (1H, m, 25-H), 3.37(3H, s, OCH_3), 3.38(3H, s, OCH_3), 3.50(1H, m, 3β-H), 3.91(1H, m, 6β-H), 4.64(2H, s, OCH_2O), 4.67 and 4.72 (each 1H, each d, J=7Hz, OCH_2O); m/z: 507(M^++1), 506(M^+), 474(M^+-CH_3OH), 445(M^+-CH_3OCH_2O).

3α,6α-Dimethoxymethyloxy-5β-cholest-22-en-24-one (34) and 3α,6α-dimethoxymethyloxy-5β-cholest-25-en-24-one (33):

The dehydrogenation reaction was carried out in the same way as **11**: the ketone **32** (160 mg, 0.32 mmol), LDA(0.63 mmol), PhSeBr (0.78 mmol) and 30% H_2O_2 (1 ml) was used. After work-up as usual compound **33** (49 mg, 30.5%) and **34** (90 mg, 56.5%) were obtained.

34 as a colorless oil: $[\alpha]_D^{24}$ +12.4°(c, 0.29, $CHCl_3$); IR(film): ν max: 1680(C=O), 1620 (C=C), 1150, 1100, 1050 cm^{-1}; ^{1}H-NMR(200MHz): 0.68(3H, s, 18-H_3), 0.92(3H, s, 19-H_3), 1.10(3H, d, J=6.6Hz, 21-H_3), 1.11(6H, d, J=6.9Hz, 26-H_3, 27-H_3), 2.24(1H, m, 20-H), 2.83 (1H, h, J=7Hz, 25-H), 3.36(3H, s, OCH_3), 3.38(3H, s, OCH_3), 3.50(1H, m, 3β-H), 3.90(1H, m, 6β-H), 4.64(2H, s, OCH_2O), 4.66 and 4.71 (each 1H, each d, J=7Hz, OCH_2O), 6.06(1H,

d, J=15.7Hz, 23-H), 6.71(1H, dd, J=8.9, 15.7Hz, 22-H); m/z: 505(M^++1), 504(M^+), 472(M^+-CH_3OH), 443(M^+-CH_3OCH_2O).

33 as a colorless oil: IR(film): ν max: 1680(C=O), 1630(C=C), 1140, 1100, 1050 cm^{-1}; ^{1}H-NMR(200MHz): 0.65(3H, s, 18-H_3), 0.92(3H, s, 19-H_3), 0.94(3H, d, J=6.4Hz, 21-H_3), 1.89(3H, d, J=0.6Hz, 25-CH_3), 2.70(2H, m, 23-H_2), 3.37(3H, s, OCH_3), 3.38(3H, s, OCH_3), 3.54(1H, m, 3β-H), 3.94(1H, m, 6β-H), 4.65(2H, s, OCH_2O), 4.68 and 4.73 (each 1H, each d, J=6.9Hz, OCH_2O), 5.77(1H, d, J=0.7Hz, 26-Hb), 5.97(1H, s, 26-Ha); m/z: 504(M^+), 443 (M^+-CH_3OCH_2O), 442(M^+-CH_3OCH_2OH).

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