Synthesis and Anti-tumor Activity of New Steroidal Nuclear Analogues of Aragusterol A

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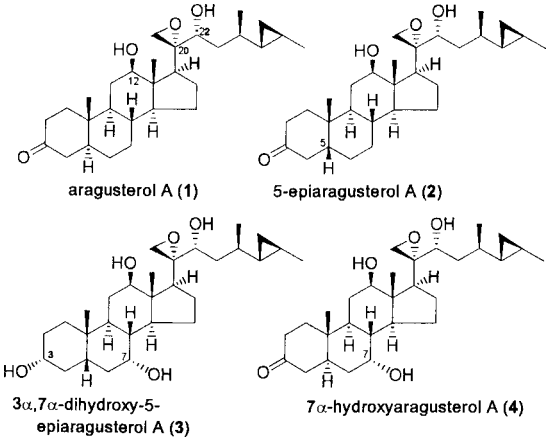
3α,7α-Dihydroxy-5-epiaragusterol A (3) was synthesized from bile acid (cholic acid) as a new steroidal nuclear analogue of antitumor marine steroid aragusterol A. 7α-Hydroxyaragusterol A (4) was also derived from xestokerol B. The *in vitro* anti-proliferative activity of each of these analogues toward KB cells as well as *in vivo* anti-tumor activity of 5-epiaragusterol A (2) previously synthesized by the authors and 3 were assessed.

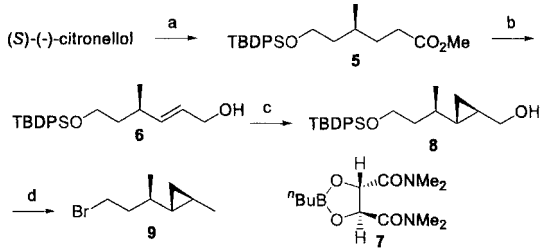
Key words synthesis; anti-tumor; steroid; analogue; aragusterol A

Aragusterol A (**1**) having a structurally unique marine steroid with a 12β-hydroxy group and rare 26,27-cyclo structure, was isolated from an Okinawan marine sponge of the genus *Xestospongia*.[1—6] This compound has been found to strongly inhibit KB cell proliferation when present at IC_{50} 0.042 μg/ml and express potent anti-tumor activity toward L1210 leukemia in mice (T/C 220% at 1.6 mg/kg).[1] A comparison of the activity of this compound with that of other aragusterols and their derivatives, indicated the configurations of C-12, C-22 hydroxy and C-20 epoxy groups to be significantly essential for expression of the anti-tumor activity of aragusterol A. A study on the underlying mechanism for inhibition of cellular proliferation has disclosed aragusterol A to arrest human non-small-cell lung-cancer (NSCLC) cells in the late G_1 phase of the cell cycle by inhibiting pRb phosphorylation.[7] The synthesis of this compound was previously carried out *via* the coupling of a steroidal nuclear segment obtained from (+)-hecogenin with the side-chain segment produced in an enantioselective manner.[8] The large scale synthesis of aragusterol A has proven difficult owing to the scarcity of (+)-hecogenin in nature. The synthesis of 5-epiaragusterol A (**2**) was thus previously carried out from bile acid (deoxycholic acid) abundantly present in nature and the compound was noted to express strong anti-proliferative activity at IC_{50} 0.041 μg/ml toward KB cells *in vitro*, the extent being comparable to that of aragusterol A.[9] In the present study, the synthesis of 3α,7α-dihydroxy-5-epiaragusterol A (**3**) from cholic acid and that of 7α-hydroxyaragusterol A (**4**) from xestokerol B[4,5] isolated from an aragusterol A-containing marine sponge were carried out so as to obtain new steroidal nuclear analogues of aragusterol A. The *in vitro* anti-proliferative activity of **3** and **4** toward KB cells and *in vivo* antitumor activity of **2** and **3** were assessed and are discussed in the following.

Results and Discussion

New steroidal nuclear analogues of aragusterol A were synthesized in this study by a method based on previously obtained results.[8,9] Prior to these syntheses, the method for obtaining side-chain segment was improved. (*S*)-(−)-Citronellol was transformed to ester **5** by protection of the hydroxy group as *tert*-butyldiphenylsilyl (TBDPS) ether, oxidative degradation of the double bond, oxidation of resulting aldehyde and esterification of the carboxyl group (Chart 1). Ester **5** was converted to allylic alcohol **6** as follows: (1) treatment with lithium diisopropylamide (LDA) and then diphenyldisulfide to afford phenylsulfide, (2) *m*-chloroperbenzoic acid (*m*CPBA) oxidation of the sulfide to sulfoxide, (3) introduction of a carbon–carbon double bond by thermal elimination and (4) diisobutylaluminum hydride (DIBAH) reduction of the ester. Diastereoselective cyclopropanation of allylic alcohol **6** was done with Charette's dioxaborolane ligand **7**[10—13] to give alcohol **8** as the sole product. Bromide **9** was obtained from alcohol **8** by conversion of the alcohol into bromide, reduction of bromide by $LiAlH_4$, deprotection of TBDPS ether and coversion of the corresponding alcohol into bromide. By this method, side-chain segment could be produced on a large scale with greater efficiency than was





Reagents and conditions: (a) 1) TBDPSCl, Et_3N, DMAP, 1,2-dichloroethane, 99%; 2) O_3, $CHCl_3$-MeOH then Me_2S; 3) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^tBuOH-H_2O; 4) MeI, DBU, CH_3CN, 3 steps 69%; (b) 1) LDA then PhSSPh, THF, 95%; 2) *m*CPBA, CH_2Cl_2; 3) toluene, reflux, 2 steps 89%; 4) DIBAH, CH_2Cl_2, 96%; (c) CH_2I_2, Et_2Zn, **7**, hexane, 99%; (d) 1) CBr_4, Ph_3P, CH_2Cl_2; 2) $LiAlH_4$, Et_2O-THF (2:1); 3) $^nBu_4NF$, THF, 3 steps 88%; 4) Ph_3P, NBS, CH_2Cl_2, 75%.

Chart 1

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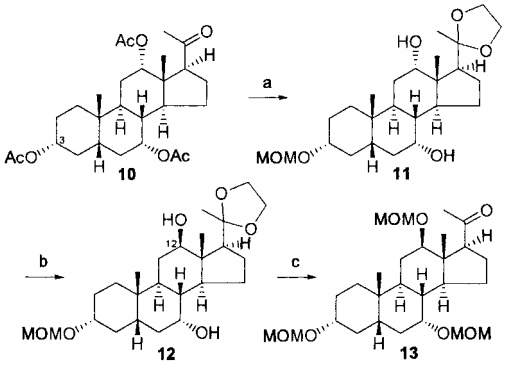
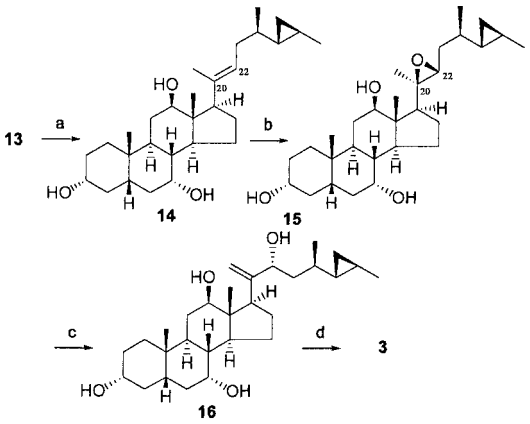


Reagents and conditions: (a) 1) ethyleneglycol, *cat.*TsOH, benzene, 73%; 2) K_2CO_3, MeOH, 95%; 3) MOMCl, $^iPr_2NEt$, CH_2Cl_2, quant; 4) K_2CO_3, MeOH, reflux, 73%; (b) 1) PCC, 4ÅMS, CH_2Cl_2, 89%; 2) L-Selectride®, THF, 53%; (c) 1) AcOH - H_2O (4:1); 2) MOMCl, $^iPr_2NEt$, CH_2Cl_2, 2 steps 68%.

Chart 2



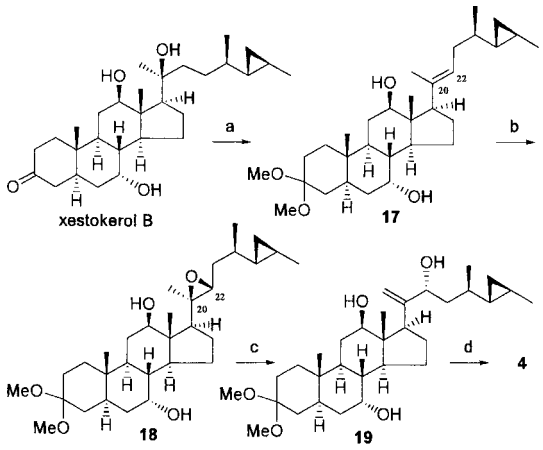
Reagents and conditions: (a) 1) **9**, lithium naphthalenide, THF; 2) *c* HCl, MeOH, 2 steps 62%; (b) ^{t}BuOOH, $VO(acac)_2$, benzene, 65%; (c) $^iPr_2NMgBr$, THF, 70%; (d) *m*CPBA, CH_2Cl_2, 88%.

Chart 3

possible previously.[8)]

From ketone **10**, obtained by degradation of the side-chain from cholic acid,[14,15)] diol **11** was produced *via* the following steps: (1) protection of the ketone as a ketal, (2) selective methanolysis of acetate at C-3 by K_2CO_3 in MeOH, (3) protection of the hydroxy group as methoxymethyl (MOM) ether and (4) deprotection of acetates by K_2CO_3 in MeOH under reflux (Chart 2). Diol **11** thus obtained was treated with pyridinium chlorochromate (PCC) to give diketone in 89% yield. Treatment of this diketone with L-Selectride® afforded diol **12** having the 12β-hydroxy group along with the 12α-isomer **11** in a 5 : 2 ratio.[16)] Deprotection of the ketal followed by protection of the hydroxy groups as MOM ether gave nuclear segment **13**.

Reaction of nuclear segment **13** with alkyllithium, obtained from bromide **9** with lithium naphthalenide,[17,18)] followed by treatment with conc. HCl gave 20(22)*E*-olefin **14** and the 20(22)*Z*-isomer in a 12 : 1 ratio (Chart 3).[19)] Stereoselective epoxidation of olefin **14** was carried out by its treatment with ^{t}BuOOH in the presence of vanadium (III) acetylacetonate ($VO(acac)_2$)[20)] to afford (20*R*,22*R*)-epoxide **15** along with (20*S*,22*S*)-isomer in a 13 : 1 ratio.[21)] Allylic alcohol **16** was derived from epoxide **15** by treatment with



Reagents and conditions: (a) *cat.* TsOH, MeOH, 54%; (b) ^{t}BuOOH, $VO(acac)_2$, benzene, 75%; (c) $^iPr_2NMgBr$, THF, 94%; (d) 1) *m*CPBA, CH_2Cl_2, 78%; 2) *cat.* PPTS, acetone, 84%.

Chart 4

$^iPr_2NMgBr$.[22)] Finally, epoxidation of allylic alcohol **16** with *m*CPBA gave 3α,7α-dihydroxy-5-epiaragusterol A (**3**) as a single product.[23)]

Treatment of xestokerol B with *p*-toluenesulfonic acid (TsOH) in MeOH provided 20(22)*E*-olefin **17** along with the 20(22)*Z*-isomer in a 4 : 1 ratio (Chart 4).[19)] Epoxidation of olefin **17** was conducted with ^{t}BuOOH in the presence of $VO(acac)_2$, giving (20*R*,22*R*)-epoxide **18** along with (20*S*,22*S*)-isomer in a 25 : 1 ratio.[21)] Epoxide **18** was converted to allylic alcohol **19** by treatment with $^iPr_2NMgBr$. Allylic alcohol **19** was made to undergo epoxidation[23)] and on deprotection of the ketal, 7α-hydroxyaragusterol A (**4**) was obtained as the sole product.

3α,7α-Dihydroxy-5-epiaragusterol A (**3**) and 7α-hydroxyaragusterol A (**4**) were found to inhibit KB cellular growth when present at IC_{50} 1.241 and 0.048 μg/ml *in vitro*, respectively. 5-Epiaragusterol A (**2**) and 3α,7α-dihydroxy-5-epiaragusterol A (**3**) expressed anti-tumor activity toward L1210 leukemia in mice (T/C 152% at 7.25 mg/kg and 205% at 25 mg/kg, respectively). *In vitro*, the lesser activity of 3α,7α-dihydroxy-5-epiaragusterol A (**3**) and greater effective dose requirement of 5-epiaragusterol A (**2**) and **3** compared to aragusterol A (**1**) may possibly owe to differences in the physical properties of these compounds. *In vivo* assessment of 7α-hydroxyaragusterol A (**4**) activity has not been possible owing to the its scarcity of this compound.

Conclusion

The authors carried out the synthesis of 3α,7α-dihydroxy-5-epiaragusterol A (**3**) from cholic acid and 7α-hydroxyaragusterol A (**4**) from xestokerol B as new steroidal nuclear analogues of aragusterol A. 3α,7α-Dihydroxy-5-epiaragusterol A (**3**) and previously synthesized 5-epiaragusterol A (**2**) were clearly shown to express *in vitro* anti-proliferative and antitumor activity. It would thus appear that the steroidal nucleus functions as determinant only of the physical properties of the analogues of aragusterol A, not its biological activity. Modification of the steroidal nucleus of aragusterol A may possibly prove useful for synthesizing analogues of aragusterol A possessing greater medical potential.

Experimental

Melting points were measured on Yazawa BY-2 micro melting point apparatus without correction. Optical rotation was measured with a JASCO DIP-360 automatic polarimeter. Infrared (IR) spectra were recorded with a Perkin-Elmer FT-IR 1710 or a JASCO FT/IR-620 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Gemini-300, a Bruker AM-400 or DPX-400. Chemical shifts were expressed on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Electron ionization mass spectra (EI-MS), electrospray ionization mass spectra (ESI-MS), high-resolution electron ionization mass spectra (HR-EI-MS) and high-resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained with a Hitachi M-80, a Thermo Quest TSQ 700, a VG Auto Spec E or a Micromass LCT spectrometer.

Methyl (*S*)-6-(*tert*-Butyldiphenylsiloxy)-4-methylhexanoate (5) To a solution of (*S*)-(−)-citronellol (5.00 g, 32.0 mmol, >99% ee) in 1,2-dichloroethane (32 ml) were added triethylamine (11.0 ml, 77.0 mmol), 4-dimethylaminopyridine (DMAP, 390 mg, 3.20 mmol) and TBDPSCl (10.0 ml, 38.0 mmol). The mixture was stirred at room temperature for 8 h. The reaction mixture was diluted with Et_2O (200 ml) and washed successively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (9 : 1) to give TBDPS ether (12.6 g, 99% yield) as a colorless oil: $[\alpha]_D^{26}$ +2.7° (*c*=4.1, $CHCl_3$). EI-MS *m/z*: 394 (M^+). HR-EI-MS *m/z*: 394.2692 (Calcd for $C_{26}H_{38}OSi$: 394.2692). IR (neat) cm^{-1}: 2930. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.86 (3H, d, *J*=6.5 Hz), 1.08 (9H, s), 1.17 (1H, m), 1.36 (2H, m), 1.62 (3H, s), 1.63 (2H, m), 1.71 (3H, s), 1.99 (2H, m), 3.71 (2H, m), 5.11 (1H, m), 7.42 (6H, m), 7.71 (4H, m).

To a solution of the above TBDPS ether (24.0 g, 60.8 mmol) in $CHCl_3$–MeOH (1 : 1, 600 ml) was added $NaHCO_3$ (16.0 g, 182 mmol). The mixture was stirred at −15 °C with introducing ozone until pale blue color persisted. Excess ozone was removed by a flow of argon. The reaction mixture was treated with Me_2S (14.0 ml, 182 mmol), allowed to worm slowly to room temperature over 2 h. The reaction mixture was stirred for 8 h at room temperature and concentrated under reduced pressure. The residue was diluted with Et_2O (100 ml) and washed successively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give crude aldehyde. The crude product was used for subsequent reaction without further purification.

To a solution of the above crude aldehyde and 2-methyl-2-butene (30.0 ml, 269 mmol) in *tert*-butanol (240 ml) were added dropwise aqueous (60 ml) solution of $NaClO_2$ (19.0 g, 209 mmol) and NaH_2PO_4 (9.30 g, 60.0 mmol) with vigorous stirring. The mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with Et_2O (600 ml) and washed successively with 30% NaH_2PO_4 solution and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give crude carboxylic acid. The crude product was used for subsequent reaction without further purification.

To a solution of the above crude carboxylic acid in CH_3CN (300 ml) were added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 36.0 ml, 240 mmol) and CH_3I (7.50 ml, 120 mmol). The mixture was stirred at room temperature for 8 h. The reaction mixture was diluted with Et_2O (150 ml) and washed successively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (9 : 1) to give ester **5** (16.7 g, 3 steps 69% yield) as a colorless oil: $[\alpha]_D^{26}$ +1.1° (*c*=3.0, $CHCl_3$). EI-MS *m/z*: 383 (M^+-CH_3). HR-EI-MS *m/z*: 383.2018 (Calcd for $C_{23}H_{31}O_3Si$: 383.2043). IR (neat) cm^{-1}: 2931, 1741. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.84 (3H, d, *J*=6.4 Hz), 1.04 (9H, s), 1.36 (1H, m), 1.46 (1H, m), 1.62 (2H, m), 2.29 (2H, m), 3.66 (3H, s), 3.70 (2H, m), 7.40 (6H, m), 7.67 (4H, m).

(*R*,*E*)-6-(*tert*-Butyldiphenylsiloxy)-4-methylhex-2-en-1-ol (6) To a cold (−78 °C) solution of diisopropylamine (45.0 ml, 328 mmol) in tetrahydrofuran (THF, 300 ml) was added ^{n}BuLi (181 ml, 280 mmol, 1.55 M in hexane). The mixture was stirred for 1 h, treated with a solution of ester **5** (93.0 g, 233 mmol) in THF (400 ml), and stirred for further 1 h. The mixture was transferred to a solution of diphenyldisulfide (62.0 g, 284 mmol) in THF (300 ml) *via* cannula. The reaction mixture was stirred at room temperature for 30 min, diluted with Et_2O (1500 ml) and washed successively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (9 : 1) to give crude sulfide. The crude product was used for subsequent reaction without further purification.

To a solution of the above crude sulfide in $CHCl_3$ (1200 ml) was added Na_2HPO_4 (32.0 g, 223 mmol). The cold (−40 °C) suspension was carefully treated with a solution of *m*CPBA (66.0 g, 268 mmol) in $CHCl_3$ (600 ml). The reaction mixture was allowed to warm slowly to room temperature, stirred for 2 h at this temperature, filtered through celite, and then concentrated under reduced pressure. The residue was dissolved in Et_2O (600 ml) and washed successively with water and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. Resulting crude sulfoxide was used for subsequent reaction without further purification.

The solution of the above crude sulfoxide in toluene (500 ml) was refluxed for 2 h and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (9 : 1) to give α,β-unsaturated ester (78.0 g, 3 steps 85% yield) as a colorless oil: $[\alpha]_D^{30}$ −16.0° (*c*=1.4, $CHCl_3$). ESI-MS *m/z*: 419 (M^++Na). HR-ESI-MS *m/z*: 419.2058 (Calcd for $C_{24}H_{32}O_3NaSi$: 419.2018). IR (neat) cm^{-1}: 3072, 1729, 1659. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.03 (3H, d, *J*=6.8 Hz), 1.04 (9H, s), 1.60 (2H, m), 2.59 (1H, m), 3.64 (1H, d, *J*=6.1 Hz), 3.67 (1H, d, *J*=6.3 Hz), 3.73 (3H, s), 5.78 (1H, dd, *J*=15.7, 1.1 Hz), 6.87 (1H, dd, *J*=15.7, 7.8 Hz), 7.40 (6H, m), 7.65 (4H, m).

To a cold (−78 °C) solution of the above α,β-unsaturated ester (1.20 g, 2.90 mmol) in CH_2Cl_2 (10 ml) was added DIBAH (7.0 ml, 6.40 mmol, 0.93 M in hexane). The reaction mixture was stirred for 30 min, diluted with Et_2O (300 ml), treated with saturated NaCl solution (10 ml) and then stirred vigorously for further 30 min. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (2 : 1) to give allylic alcohol **6** (1.00 g, 97% yield) as a colorless oil: $[\alpha]_D^{30}$ −7.8° (*c*=0.8, $CHCl_3$). ESI-MS *m/z*: 391 (M^++Na). HR-ESI-MS *m/z*: 391.2057 (Calcd for $C_{23}H_{32}O_2NaSi$: 391.2069). IR (neat) cm^{-1}: 3327, 2930. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.97 (3H, d, *J*=6.8 Hz), 1.04 (9H, s), 1.34 (1H, m), 1.56 (2H, m), 2.37 (1H, m), 3.65 (2H, t, *J*=10.5 Hz), 4.03 (2H, d, *J*=4.6 Hz), 5.53 (2H, m), 7.43 (6H, m), 7.66 (4H, m).

{2-[(*R*)-4-(*tert*-Butyldiphenylsiloxy)butyl]-(1*R*,2*S*)-cyclopropyl}-methanol (8) To the cold solution (0 °C) of Et_2Zn (8.40 ml, 8.40 mmol, 1.0 M in hexane) in CH_2Cl_2 (30 ml) was carefully added CH_2I_2 (1.40 ml, 16.7 mmol) with vigorous stirring.[24] The mixture was stirred at 0 °C for 30 min, and a preformed solution of dioxaborolane **7** (25.0 ml, 2.50 mmol, 0.1 M in CH_2Cl_2) and allylic alcohol **6** (770 mg, 2.10 mmol) was added *via* cannula. The resulting mixture was stirred at room temperature for 3 h, diluted with Et_2O (200 ml), washed successively with saturated NH_4Cl solution, water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (3 : 2) to give alcohol **8** (795 mg, 99% yield) as a colorless oil: $[\alpha]_D^{26}$ −8.0° (*c*=1.5, $CHCl_3$). ESI-MS *m/z*: 405 (M^++Na). HR-ESI-MS *m/z*: 405.2229 (Calcd for $C_{24}H_{34}O_2NaSi$: 405.2226). IR (neat) cm^{-1}: 3354, 2998. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.31 (2H, m), 0.40 (1H, m), 0.88 (1H, m), 0.91 (3H, d, *J*=6.3 Hz), 0.99 (1H, m), 1.04 (9H, s), 1.52 (1H, dq, *J*=13.4, 6.7 Hz), 1.66 (1H, dq, *J*=13.4, 6.7 Hz), 3.53 (2H, m), 3.72 (1H, dd, *J*=10.3, 6.7 Hz), 7.40 (6H, m), 7.68 (4H, m).

(*R*)-1-Bromo-3-[(1*R*,2*R*)-2-methylcyclopropyl]butane (9) To a solution of alcohol **8** (607 mg, 1.60 mmol) in CH_2Cl_2 (5.0 ml) were added Ph_3P (500 mg, 1.90 mmol) and CBr_4 (630 mg, 1.90 mmol). The mixture was stirred at room temperature for 10 min, diluted with Et_2O (50 ml), filtered through silica gel, and concentrated under reduced pressure. Resulting crude bromide was used for subsequent reaction without further purification.

To a cold (0 °C) solution of the above crude bromide in Et_2O–THF (2 : 1, 5.10 ml) was carefully added $LiAlH_4$ (90.0 mg, 2.40 mmol). The mixture was stirred at room temperature for 1 h, diluted with Et_2O (50 ml), treated with saturated NaCl solution (2.0 ml) and then stirred vigorously for further 30 min. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Resulting crude TBDPS ether was used for subsequent reaction without further purification.

To a solution of the above crude TBDPS ether in THF (5.0 ml) was added ^{n}Bu$_4$NF (2.40 ml, 2.40 mmol, 1.0 M in THF). The mixture was stirred at room temperature for 1 h, diluted with Et_2O (50 ml), washed successively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with pentane–Et_2O (1 : 1) to give alcohol (180 mg, 3 steps 88% yield) as a colorless oil: $[\alpha]_D^{29}$ −36.6° (*c*=1.3, $CHCl_3$). EI-MS *m/z*: 110 (M^+-H_2O). HR-EI-MS *m/z*: 110.1084 (Calcd for C_8H_{14}: 110.1096). IR (neat) cm^{-1}: 3388, 2994. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.16 (3H, m), 0.46 (1H, m), 0.85 (1H, m), 0.93 (3H, d, *J*=6.3 Hz), 1.00 (3H, d, *J*=5.8 Hz), 1.63 (1H, dq, *J*=13.8, 6.9 Hz), 1.65 (1H, dq, *J*=13.8, 6.9 Hz), 3.72 (2H, t, *J*=6.9 Hz).

To a solution of the above alcohol (2.60 g, 20.1 mmol) in CH_2Cl_2 (100 ml) were added Ph_3P (7.90 g, 30.2 mmol) and *N*-bromosuccinimide (NBS, 5.40 g, 30.2 mmol). The mixture was stirred at room temperature for 10 min, diluted with Et_2O (500 ml), filtered through silica gel, and concentrated under reduced pressure. The residue was distilled (50 °C, 3 mmHg) to give bromide **9** (2.60 g, 13.5 mmol, 67% yield) as a colorless oil: $[\alpha]_D^{23}$ −26.4° (c=0.9, $CHCl_3$). EI-MS *m/z*: 190 (M^+). HR-EI-MS *m/z*: 190.0355 (Calcd for $C_8H_{15}Br$: 190.0357). IR (neat) cm^{-1}: 2875. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.13 (2H, d, J=4.7 Hz), 0.20 (1H, d, J=8.6 Hz), 0.57 (1H, m), 0.95 (3H, s), 1.02 (3H, d, J=6.0 Hz), 1.90 (2H, m), 3.51 (2H, t, J=7.5 Hz).

7α,12α-Dihydroxy-3α-[(methoxymethyl)oxy]-5β-pregnan-20-one Ethylene Ketal (11) To a solution of 3α,7α,12α-triacetoxy-5β-pregnan-20-one[8] (**10**, 1.00 g, 2.20 mmol) in benzene (25 ml) were added ethylene glycol (250 μl, 4.30 mmol) and TsOH (20 mg). The mixture was stirred for 2 h under reflux to remove the H_2O produced in the reaction (Dean-Stark trap). After addition of pyridine (100 μl) at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–EtOAc (1 : 1) to give triacetate (802 mg, 73% yield) as a colorless amorphous: $[\alpha]_D^{24}$ +86.7° (c=0.6, $CHCl_3$). EI-MS *m/z*: 505 (M^+-CH_3). HR-EI-MS *m/z*: 505.2816 (Calcd for $C_{28}H_{41}O_8$: 505.2801). IR (KBr) cm^{-1}: 2942, 1735, 1249. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.81 (3H, s), 0.90 (3H, s), 1.19 (3H, s), 2.03 (3H, s), 2.07 (3H, s), 2.11 (3H, s), 2.27 (1H, br t, J=9.9 Hz), 3.87 (4H, m), 4.56 (1H, m), 4.90 (1H, m), 5.08 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 13.2, 21.4, 21.5, 21.6, 22.3, 22.4, 22.5, 22.6, 24.0, 25.2, 26.9, 29.0, 31.2, 34.4, 34.6, 37.2, 40.9, 43.2, 44.6, 49.7, 63.3, 64.8, 70.7, 74.1, 74.9, 111.2, 170.3, 170.5, 170.6.

To a solution of the above triacetate (100 mg, 0.19 mmol) in MeOH (2.0 ml) was added K_2CO_3 (30.0 mg, 0.21 mmol). The mixture was stirred at room temperature for 3 h and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc to give 3α-alcohol (85.0 mg, 95% yield) as a colorless amorphous: $[\alpha]_D^{23}$ +73.8° (c=0.4, $CHCl_3$). EI-MS *m/z*: 463 (M^+-CH_3). HR-EI-MS *m/z*: 463.2682 (Calcd for $C_{26}H_{39}O_7$: 463.2696). IR (KBr) cm^{-1}: 3413, 2931, 1733, 1245. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.80 (3H, s), 0.89 (3H, s), 1.15 (3H, s), 2.06 (3H, s), 2.10 (3H, s), 2.30 (1H, br t, J=10.1 Hz), 3.48 (1H, m), 3.86 (4H, m), 4.89 (1H, m), 5.07 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 13.2, 21.5, 21.6, 22.3, 22.4, 22.5, 24.0, 25.2, 29.0, 30.4, 31.3, 34.3, 34.8, 37.2, 38.6, 41.0, 43.2, 44.6, 49.6, 63.2, 64.7, 70.8, 71.6, 74.9, 111.2, 170.6, 170.7.

To a solution of the above alcohol (3.80 g, 8.00 mmol) in CH_2Cl_2 (40 ml) were added ^{i}Pr$_2$NEt (2.30 ml, 16.1 mmol) and MOMCl (0.92 ml, 12.1 mmol). The mixture was stirred for 3 h at room temperature, diluted with Et_2O (200 ml), washed successively with water and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (1 : 1) to give 7α,12α-diacetate (4.20 mg, >99% yield) as a colorless amorphous: $[\alpha]_D^{27}$ +65.1° (c=0.7, $CHCl_3$). EI-MS *m/z*: 507 (M^+-CH_3). HR-EI-MS *m/z*: 507.2944 (Calcd for $C_{28}H_{43}O_8$: 507.2958). IR (KBr) cm^{-1}: 2934, 1734, 1249. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.80 (3H, s), 0.88 (3H, s), 1.17 (3H, s), 2.05 (3H, s), 2.07 (3H, s), 2.30 (1H, br t, J=9.9 Hz), 3.34 (3H, s), 3.37 (1H, m), 3.86 (4H, m), 4.65 (2H, s), 4.89 (1H, m), 5.07 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 13.2, 21.4, 21.5, 22.2, 22.4, 22.5, 22.6, 24.0, 25.2, 27.9, 29.0, 31.4, 34.5, 34.9, 36.0, 37.2, 41.2, 43.2, 44.5, 49.6, 55.1, 63.2, 64.7, 70.8, 74.9, 94.7, 111.2, 170.5, 170.7.

To a solution of the above diacetate (4.10 g, 7.90 mmol) in MeOH (80 ml) was added K_2CO_3 (5.50 g, 39.5 mmol). The mixture was stirred under reflux for 3 d and concentrated under reduced pressure. The residue was dissolved in Et_2O (500 ml), washed successively with water and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc to give 7α,12α-diol **11** (2.50 g, 73% yield) as a colorless amorphous: $[\alpha]_D^{31}$ +33.6° (c=0.3, $CHCl_3$). EI-MS *m/z*: 423 (M^+-CH_3). HR-EI-MS *m/z*: 423.2743 (Calcd for $C_{24}H_{39}O_6$: 423.2747). IR (KBr) cm^{-1}: 3473, 2935. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.77 (3H, s), 0.87 (3H, s), 1.27 (3H, s), 2.32 (1H, br t, J=10.8 Hz), 3.34 (3H, s), 3.35 (1H, m), 3.82 (1H, m), 3.92 (5H, m), 4.65 (2H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.9, 22.7, 22.8, 23.0, 23.9, 27.4, 27.6, 27.8, 34.7, 35.1, 35.4, 36.7, 39.2, 41.8, 42.1, 46.3, 49.8, 55.2, 63.8, 64.3, 68.4, 72.4, 77.5, 94.4, 112.2.

7α,12β-Dihydroxy-3α-[(methoxymethyl)oxy]-5β-pregnan-20-one Ethylene Ketal (12) To a solution of 7α,12α-diol **11** in 1,2-dicholoroethane (100 ml) were added molecular sieves (4A, powder, 6.50 g) and PCC (6.50 g, 17.1 mmol). The mixture was stirred at room temperature for 4 h, diluted with Et_2O (200 ml), filtered through silica gel, and concentrated under reduced pressure to afford 7,12-diketone (2.20 g, 89% yield) as a colorless amorphous: $[\alpha]_D^{31}$ +35.9° (c=0.3, $CHCl_3$). EI-MS *m/z*: 419 (M^+-CH_3). HR-EI-MS *m/z*: 419.2431 (Calcd for $C_{24}H_{35}O_6$: 419.2434). IR (KBr) cm^{-1}: 2937, 1709. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.10 (3H, s), 1.27 (3H, s), 1.27 (3H, s), 1.90 (1H, m), 1.99 (1H, dd, J=13.0, 2.1 Hz), 2.06 (1H, dd, J=12.5, 5.2 Hz), 2.24 (1H, m), 2.70 (1H, t, J=12.9 Hz), 2.70 (1H, dd, J=10.2, 9.4 Hz), 2.77 (1H, br t, J=11.7 Hz), 2.87 (1H, dd, J=12.9, 6.0 Hz), 3.31 (3H, s), 3.46 (1H, m), 3.82 (1H, q, J=7.0 Hz), 3.84 (1H, q, J=7.4 Hz), 3.93 (1H, q, J=7.4 Hz), 3.97 (1H, q, J=7.0 Hz), 4.60 (2H, br s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 13.0, 22.1, 22.5, 24.5, 24.7, 27.0, 34.0, 34.5, 35.9, 37.9, 45.1, 45.4, 48.3, 48.4, 51.5, 55.2, 56.5, 62.9, 65.4, 75.4, 94.6, 111.1, 209.2, 211.6.

To a cold (0 °C) solution of the above 7,12-diketone (100 mg, 0.23 mmol) in THF (2.0 ml) was added dropwise L-Selectride® (0.7 ml, 0.7 mmol, 1.0 M in THF). The mixture was stirred at room temperature for 2 h, treated with 1 N NaOH solution and 30% H_2O_2 solution, and stirred further 30 min. The mixture was diluted with Et_2O (20 ml), washed successively with saturated NH_4Cl solution, water and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc to give 7α,12β-diol **12** (53.2 mg, 52% yield) along with 12α isomer **11** (22.2 mg, 22% yield): 7α,12β-diol **12**: colorless amorphous. $[\alpha]_D^{26}$ +14.3° (c=1.8, $CHCl_3$). EI-MS *m/z*: 423 (M^+-CH_3). HR-EI-MS *m/z*: 423.2730 (Calcd for $C_{24}H_{39}O_6$: 423.2747). IR (KBr) cm^{-1}: 3495, 3435, 2937. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.75 (3H, s), 0.90 (3H, s), 1.31 (3H, s), 2.18 (1H, br t, J=11.1 Hz), 3.35 (3H, s), 3.36 (1H, m), 3.86 (1H, m), 4.01 (5H, m), 4.67 (2H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 7.8, 22.6, 23.1, 23.4, 25.4, 27.6, 28.0, 31.7, 35.0, 35.2, 35.2, 36.6, 37.6, 41.2, 48.1, 48.8, 55.1, 57.5, 63.3, 63.7, 68.0, 76.7, 77.9, 94.3, 111.5.

3α,7α,12β-Tri[(methoxymethyl)oxy]-5β-pregnan-20-one (13) A solution of 7α,12β-diol **12** (1.20 g, 2.70 mmol) in 80% acetic acid was stirred at room temperature for 2 h, and then concentrated under reduced pressure. Resulting crude 7,12-dihydroxyketone was used for subsequent reaction without further purification.

To a solution of the above 7,12-dihydroxyketone in 1,2-dichloroethane (15 ml) were added ^{i}Pr$_2$NEt (3.80 ml, 21.6 mmol) and MOMCl (1.20 ml, 16.2 mmol). The mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with Et_2O (100 ml), and washed successively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (1 : 2) to give ketone **13** (900 mg, 2 steps 69% yield) as a colorless viscous oil. $[\alpha]_D^{27}$+42.5° (c=1.35, $CHCl_3$). EI-MS *m/z*: 482 (M^+). HR-EI-MS *m/z*: 451.3047 (Calcd for $C_{26}H_{43}O_6$: 451.3060). IR (KBr) cm^{-1}: 2938, 1707. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.72 (3H, s), 0.91 (3H, s), 0.99 (1H, dt, J=14.1, 2.9 Hz), 1.91 (1H, dt, J=13.0, 4.8 Hz), 2.20 (3H, s), 2.73 (1H, br t, J=8.7 Hz), 3.30 (3H, s), 3.34 (3H, s), 3.35 (3H, s), 3.35 (1H, m), 3.42 (1H, dd, J=10.9, 4.6 Hz), 3.65 (1H, m), 4.68 (6H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 8.8, 22.6, 24.0, 26.0, 27.0, 27.8, 30.7, 32.2, 32.3, 35.3, 35.4, 36.2, 38.5, 41.4, 49.3, 50.2, 55.1, 55.6, 56.1, 61.6, 74.3, 77.0, 86.0, 94.6, 95.4, 96.0, 211.3.

(20*E*,24*R*,25*R*,26*R*)-26,27-Cyclo-3α,7α,12β-trihydroxy-24,26-dimethyl-5β-cholest-20(22)-ene (14) To a solution of naphthalene (1.50 g, 11.7 mmol) in THF (20 ml) was added Li (70.0 mg, 10.0 mmol). The mixture was stirred at room temperature to become a homogeneous solution. Bromide **9** (1.00 g, 5.00 mmol) was added to the mixture at −78 °C. After stirring for 30 min, a solution of ketone **13** (800 mg, 1.66 mmol) in THF (9.00 ml) was added dropwise to the mixture. The reaction mixture was stirred for 30 min at −78 °C and then allowed to warm slowly to room temperature. Reaction mixture was diluted with Et_2O (150 ml), and washed successively with saturated NH_4Cl solution, water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Resulting crude product was used for subsequent reaction without further purification.

To a solution of the above crude product in MeOH (8.0 ml) was added TsOH (8.0 mg). The mixture was stirred for 12 h under reflux. The reaction mixture was added pyridine (0.5 ml), and then concentrated under reduced pressure. The residue was chromatographed on a silica gel column with Et_2O to give 20(22)*E*-olefin **14** (450 mg, 2 steps 60% yield) and 20(22)*Z*-isomer (35.0 mg, 2 steps 5% yield).

20(22)*E*-Olefin **14**: Colorless amorphous. $[\alpha]_D^{28}$−36.6° (c=0.1, $CHCl_3$). EI-MS *m/z*: 444 (M^+). HR-EI-MS *m/z*: 444.3592 (Calcd for $C_{29}H_{48}O_3$: 444.3604). IR (KBr) cm^{-1}: 3424, 2951. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.11 (1H, m), 0.18 (2H, m), 0.47 (1H, m), 0.75 (3H, s), 0.91 (3H, s), 0.92 (3H, d, J=6.6 Hz), 0.98 (3H, d, J=6.0 Hz), 1.70 (3H, br s), 2.37 (1H, t, J=9.9 Hz), 3.46 (1H, m), 3.65 (1H, dd, J=11.3, 5.0 Hz), 3.88 (1H, m), 5.56 (1H, br t, J=7.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 8.6, 11.8, 12.7, 15.3, 19.0, 19.8, 22.6, 23.7, 25.2, 26.9, 28.2, 30.6, 32.0, 34.8, 35.1, 35.3, 35.9, 38.2, 39.0, 39.8, 41.2, 47.7, 49.1, 60.8, 68.2, 71.8, 80.0, 127.6, 139.3.

(20*R*,22*R*,24*R*,25*R*,26*R*)-26,27-Cyclo-20,22-epoxy-24,26-dimethyl-5β-cholestane-3α,7α,12β-triol (15) To a solution of 20(22)*E*-olefin **14** (435 mg, 0.98 mmol) in benzene (5 ml) were added $VO(acac)_2$ (3.00 mg, 0.01 mmol) and *tert*-butylhydroperoxide[25] (TBHP, 0.50 ml, 1.47 mmol, 3.0 M in CH_2Cl_2). The mixture was stirred for 3 d at room temperature. To the mixture was added Me_2S (0.5 ml) and stirred for 1 h. The reaction mixture was diluted with Et_2O (40 ml), and washed successively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc to give (20*R*,22*R*)-epoxide **15** (293 mg, 65% yield) and (20*S*,22*S*)-isomer (22.0 mg, 5% yield).

(20*R*,22*R*)-Epoxide **15**: Colorless amorphous. $[\alpha]_D^{26}$ −10.8° (*c*=1.1, $CHCl_3$). EI-MS *m/z*: 460 (M^+). HR-EI-MS *m/z*: 460.3566 (Calcd for $C_{29}H_{48}O_4$: 460.3553). IR ($CHCl_3$) cm^{-1}: 3412, 2951. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.14 (1H, m), 0.22 (2H, m), 0.53 (1H, m), 0.70 (3H, s), 0.88 (3H, s), 1.00 (3H, d, *J*=6.5 Hz), 1.02 (3H, d, *J*=6.0 Hz), 1.30 (3H, s), 3.32 (1H, dd, *J*=11.2, 4.6 Hz), 3.40 (1H, t, *J*=6.1 Hz), 3.43 (1H, m), 3.85 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 9.0, 12.0, 12.8, 19.1, 20.0, 20.1, 22.6, 22.8, 26.3, 26.9, 28.2, 30.7, 31.9, 35.0, 35.1, 35.3, 36.1, 36.8, 37.7, 39.7, 41.2, 48.0, 49.1, 57.3, 61.7, 64.1, 68.0, 71.8, 76.9.

(22*R*,24*R*,25*R*,26*R*)-26,27-Cyclo-24,26-dimethyl-5β-cholest-20-ene-3α,7α,12β,22-tetraol (16) To $^iPr_2NH$ (0.91 ml, 6.50 mmol) in flask was added dropwise MeMgBr (6.0 ml, 0.93 M in THF) at room temperature. After stirring for 1 h, resulting solution was added to (20*R*,22*R*)-epoxide **15** (250 mg, 0.54 mmol) in another flask, and stirred for 3 d at room temperature. The reaction mixture was diluted with Et_2O (20 ml), and washed successively with saturated NH_4Cl solution, water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc to give allylic alcohol **16** (173 mg, 70% yield) as a colorless amorphous: $[\alpha]_D^{26}$ −1.0° (*c*=1.1, $CHCl_3$). EI-MS *m/z*: 460 (M^+). HR-EI-MS *m/z*: 460.3533 (Calcd for $C_{29}H_{48}O_4$: 460.3553). IR ($CHCl_3$) cm^{-1}: 3382, 2927, 1639. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.09 (1H, m), 0.17 (2H, m), 0.51 (1H, m), 0.75 (3H, s), 0.90 (3H, d, *J*=6.4 Hz), 0.94 (3H, s), 0.99 (3H, d, *J*=6.0 Hz), 3.44 (1H, m), 3.64 (1H, dd, *J*=11.6, 4.3 Hz), 3.88 (1H, m), 4.31 (1H, dd, *J*=8.3, 5.8 Hz), 4.91 (1H, s), 5.08 (1H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 8.0, 11.6, 12.9, 19.3, 19.6, 22.6, 23.1, 27.5, 29.9, 30.5, 31.5, 32.7, 34.3, 34.6, 34.8, 35.4, 39.0, 39.1, 41.3, 44.4, 48.0, 48.6, 50.6, 67.9, 71.5, 76.1, 79.0, 112.8, 151.2.

3α,7α-Dihydroxy-5-epiaragusterol A (3) To a cold (0 °C) solution of allylic alcohol **16** (150 mg, 0.33 mmol) in CH_2Cl_2 (1.50 ml) were added Na_2HPO_4 (140 mg, 0.98 mmol) and *m*CPBA (160 mg, 0.65 mmol). After stirring for 1 h, the mixture was treated with Me_2S (0.5 ml), and stirred for further 1 h. The mixture was diluted with Et_2O (15 ml), washed successively with water, saturated $NaHCO_3$ solution, water again and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc to give 3α,7α-dihydroxy-5-epiaragusterol A (**3**, 139 mg, 88% yield) as a colorless powder: mp 66—67 °C. $[\alpha]_D^{26}$ −1.0° (*c*=1.1, $CHCl_3$). EI-MS *m/z*: 476 (M^+). HR-EI-MS *m/z*: 476.3495 (Calcd for $C_{29}H_{48}O_5$: 476.3502). IR ($CHCl_3$) cm^{-1}: 3416, 2932. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.16 (1H, m), 0.23 (2H, m), 0.48 (1H, m), 0.68 (3H, s), 0.90 (3H, s), 0.92 (3H, br s), 1.01 (3H, d, *J*=6.0 Hz), 2.34 (1H, br t, *J*=9.6 Hz), 2.79 (1H, d, *J*=3.8 Hz), 3.04 (1H, d, *J*=3.8 Hz), 3.35 (2H, m), 3.57 (1H, dd, *J*=11.1, 4.5 Hz), 3.84 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 7.7, 12.4, 12.5, 18.8, 19.2, 22.7, 23.3, 27.4, 27.6, 28.8, 31.0, 31.9, 34.8, 35.0, 35.3, 35.4, 37.7, 39.9, 41.3, 46.5, 48.7, 48.9, 51.2, 66.0, 67.6, 71.8, 74.0, 77.4.

(20*E*,24*R*,25*R*,26*R*)-26,27-Cyclo-7α,12β-dihydroxy-24,26-dimethyl-5β-cholest-20(22)-en-3-one Dimethyl Ketal (17) To a solution of xestokerol B[4,5] (500 mg, 1.09 mmol) in MeOH (20 ml) was added TsOH (20.0 mg). The mixture was stirred for 18 h under reflux. The reaction mixture was added pyridine (1.0 ml), and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (2 : 1) to give 20(22)*E*-olefin **17** (286 mg, 54% yield) and 20(22)*Z*-isomer (71.5 mg, 13% yield).

20(22)*E*-Olefin **17**: Colorless amorphous. $[\alpha]_D^{26}$ −16.5° (*c*=0.2, $CHCl_3$). EI-MS *m/z*: 488 (M^+). HR-EI-MS *m/z*: 488.3865 (Calcd for $C_{31}H_{52}O_4$: 488.3866). IR (KBr) cm^{-1}: 3446. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.09 (1H, m), 0.16 (2H, m), 0.46 (1H, m), 0.74 (1H, m), 0.80 (3H, s), 0.91 (3H, d, *J*=6.6 Hz), 0.97 (3H, d, *J*=6.0 Hz), 1.69 (3H, br s), 3.13 (3H, s), 3.18 (3H, s), 3.58 (1H, dd, *J*=10.4, 4.5 Hz), 3.84 (1H, m), 5.55 (1H, t, *J*=6.6 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 8.6, 10.5, 11.7, 12.7, 15.3, 19.0, 19.7, 23.7, 25.2, 26.9, 28.3, 28.5, 34.6, 34.9, 35.0, 35.8, 35.9, 36.4, 38.4, 39.0, 44.6, 47.5, 47.5, 47.7, 49.0, 60.8, 67.5, 79.8, 100.2, 127.5, 139.2.

20(22)*Z*-Isomer: Colorless amorphous. $[\alpha]_D^{26}$ −20.0° (*c*=0.2, $CHCl_3$). EI-MS *m/z*: 488 (M^+). HR-EI-MS *m/z*: 488.3860 (Calcd for $C_{31}H_{52}O_4$: 488.3866). IR (KBr) cm^{-1}: 3447. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.11 (1H, m), 0.20 (2H, m), 0.49 (1H, m), 0.77 (3H, s), 0.80 (3H, s), 0.87 (3H, d, *J*=6.4 Hz), 1.00 (3H, d, *J*=6.0 Hz), 1.78 (3H, br s), 3.13 (3H, s), 3.18 (3H, s), 3.66 (1H, dd, *J*=11.0, 4.9 Hz), 3.86 (1H, m), 5.42 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 9.0, 10.5, 11.7, 12.7, 19.0, 19.2, 22.2, 23.6, 24.7, 26.8, 28.3, 28.4, 34.7, 34.9, 35.1, 35.9, 36.4, 36.6, 38.4, 39.2, 44.6, 47.5, 47.5, 48.0, 49.5, 50.4, 67.6, 79.5, 100.2, 128.5, 138.6.

(20*R*,22*R*,24*R*,25*R*,26*R*)-26,27-Cyclo-20,22-epoxy-7α,12β-dihydroxy-24,26-dimethy-l5β-cholestan-3-one Dimethyl Ketal (18) To a solution of 20(22)*E*-olefin **17** (286 mg, 0.59 mmol) in benzene (6.0 ml) were added $VO(acac)_2$ (2.00 mg, 5.90 μmol) and TBHP (0.25 ml, 0.74 mmol, 3.0 M in CH_2Cl_2). The mixture was stirred for 2 h at room temperature. The reaction mixture was treated with Me_2S (0.5 ml), and stirred for 1 h. The mixture was diluted with Et_2O (40 ml), washed successively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane–Et_2O (1 : 2) to give (20*R*,22*R*)-epoxide **18** (220 mg, 75% yield) and (20*S*,22*S*)-isomer (14.0 mg, 0.5% yield).

(20*R*,22*R*)-Epoxide **18**: Colorless amorphous. $[\alpha]_D^{26}$ −4.4° (*c*=0.3, $CHCl_3$). EI-MS *m/z*: 504 (M^+). HR-EI-MS *m/z*: 504.3801 (Calcd for $C_{31}H_{52}O_5$: 504.3815). IR ($CHCl_3$) cm^{-1}: 3399. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.14 (1H, m), 0.22 (2H, m), 0.53 (1H, m), 0.69 (3H, s), 0.77 (3H, s), 0.97 (3H, d, *J*=6.6 Hz), 1.01 (3H, d, *J*=6.0 Hz), 1.29 (3H, s), 3.11 (3H, s), 3.17 (3H, s), 3.23 (1H, dd, *J*=10.9, 4.2 Hz), 3.81 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 9.2, 10.6, 12.9, 19.2, 20.2, 20.2, 23.0, 26.5, 27.1, 28.5, 28.8, 34.8, 35.0, 35.2, 35.3, 36.0, 36.3, 36.8, 36.9, 38.1, 44.6, 47.6, 47.6, 48.2, 49.2, 57.6, 61.8, 64.1, 67.6, 77.5, 100.4.

(20*S*,22*S*)-Isomer: Colorless amorphous. $[\alpha]_D^{26}$ −12.8° (*c*=0.4, $CHCl_3$). EI-MS *m/z*: 504 (M^+). HR-EI-MS *m/z*: 504.3813 (Calcd for $C_{31}H_{52}O_5$: 504.3815). IR ($CHCl_3$) cm^{-1}: 3427. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.11 (1H, m), 0.22 (2H, m), 0.53 (1H, m), 0.81 (3H, s), 0.85 (3H, s), 0.99 (3H, d, *J*=6.6 Hz), 1.00 (3H, d, *J*=6.1 Hz), 2.85 (1H, t, *J*=7.0 Hz), 3.13 (3H, s), 3.18 (3H, s), 3.27 (1H, dd, *J*=10.8, 5.6 Hz), 3.88 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 9.3, 10.5, 11.9, 12.6, 15.7, 19.1, 20.0, 23.8, 24.7, 26.7, 26.8, 27.0, 28.4, 28.7, 34.7, 35.0, 35.1, 35.8, 36.0, 36.7, 38.5, 44.6, 47.6, 48.0, 52.3, 59.6, 61.0, 67.8, 78.0, 100.3.

(22*R*,24*R*,25*R*,26*R*)-26,27-Cyclo-7α,12β,22-trihydroxy-24,26-dimethyl-5β-cholest-20-en-3-one Dimethyl Ketal (19) To $^iPr_2NH$ (0.73 ml, 5.20 mmol) in flask was added dropwise MeMgBr (4.7 ml, 0.93 M in THF). The mixture was stirred at room temperature for 1 h. The mixture was added to a solution of (20*R*,22*R*)-epoxide **18** (219 mg, 0.43 mmol) in THF (2.0 ml), and stirred for 16 h at room temperature. The reaction mixture was diluted with Et_2O, and washed successively with saturated NH_4Cl solution, water and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with Et_2O to give allylic alcohol **19** (205 mg, 94% yield) as a colorless amorphous: $[\alpha]_D^{26}$ −21.3° (*c*=0.4, $CHCl_3$). EI-MS *m/z*: 504 (M^+). HR-EI-MS *m/z*: 504.3810 (Calcd for $C_{31}H_{52}O_5$: 504.3815). IR ($CHCl_3$) cm^{-1}: 3344. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.09 (1H, m), 0.17 (2H, m), 0.51 (1H, m), 0.71 (3H, s), 0.79 (3H, s), 0.91 (3H, d, *J*=6.6 Hz), 0.99 (3H, d, *J*=5.9 Hz), 3.11 (3H, s), 3.16 (3H, s), 3.58 (1H, dd, *J*=10.9, 4.2 Hz), 4.28 (1H, dd, *J*=13.4, 5.5 Hz), 4.89 (1H, s), 5.06 (1H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 7.9, 10.4, 11.8, 12.8, 19.1, 19.8, 23.3, 27.5, 28.2, 29.8, 31.8, 34.5, 34.7, 34.8, 35.0, 35.7, 36.3, 39.1, 44.5, 45.0, 47.4, 47.5, 47.7, 48.7, 49.7, 67.3, 76.2, 78.1, 100.3, 112.9, 151.4.

7α-Hydroxyaragusterol A (4) To a cold (0 °C) solution of allylic alcohol **19** (204 mg, 0.40 mmol) in CH_2Cl_2 (4.0 ml) were added Na_2HPO_4 (172 mg, 1.21 mmol) and *m*CPBA (200 mg, 0.81 mmol). The mixture was stirred at 0 °C for 4 h, treated with Me_2S (0.2 ml), and stirred for further 1 h. The mixture was diluted with Et_2O, and washed successively with water, saturated $NaHCO_3$ solution, water again and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with Et_2O to give 7α-hydroxyaragusterol A dimethyl ketal (165 mg, 78% yield) as a colorless amorphous: $[\alpha]_D^{26}$ +5.0° (*c*=0.2, $CHCl_3$). EI-MS *m/z*: 520 (M^+). HR-EI-MS *m/z*: 520.3779 (Calcd for $C_{31}H_{52}O_6$: 520.3764). IR ($CHCl_3$) cm^{-1}: 3403. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.15 (1H, m), 0.24 (2H, m), 0.48 (1H, m), 0.69 (3H, s), 0.78 (3H, s), 0.93 (3H, s), 1.01 (3H, d, *J*=6.0 Hz), 2.17 (1H, dd, *J*=10.7, 2.0 Hz), 2.94 (1H, d, *J*=4.8 Hz), 3.07 (1H, d, *J*=4.8 Hz), 3.13 (3H, s), 3.18 (3H, s), 3.36 (1H, dd, *J*=10.9, 4.2 Hz), 3.49 (1H, d, *J*=8.7 Hz), 3.84 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 8.1, 10.4, 12.4, 12.4, 18.8, 19.1, 23.1, 26.7, 27.6, 28.2, 28.9, 29.7, 34.6, 34.9, 35.1, 35.8, 36.6, 37.8, 40.1,

44.3, 47.5, 47.5, 48.2, 48.6, 48.8, 50.4, 65.6, 67.5, 71.8, 77.1, 100.2.

To the above ketal (144 mg, 0.28 mmol), pyridinium *p*-toluenesulfonate (PPTS, 0.1% in acetone) was added. The mixture was stirred for 4 h at room temperature. The reaction mixture was added pyridine (0.1 ml), and concentrated under reduced pressure to give crude product. The solid was recrystallized from EtOAc to provide 7α-hydroxyaragusterol A (**4**, 84.0 mg, 64% yield) as a colorless amorphous: $[\alpha]_D^{26}+18.0°$ ($c=0.2$, $CHCl_3$). EI-MS *m*/*z*: 474 (M^+). HR-EI-MS *m*/*z*: 474.3344 (Calcd for $C_{29}H_{46}O_5$: 474.3345). IR ($CHCl_3$) cm^{-1}: 3359, 1712. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.16 (1H, m), 0.25 (2H, m), 0.50 (1H, m), 0.73 (3H, s), 0.95 (3H, s), 1.00 (3H, s), 1.02 (3H, d, $J=6.0$ Hz), 2.95 (1H, d, $J=4.0$ Hz), 3.07 (1H, d, $J=4.0$ Hz), 3.39 (1H, dd, $J=10.0$, 6.9 Hz), 3.49 (1H, d, $J=8.9$ Hz), 3.88 (1H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 8.0, 10.3, 12.4, 12.4, 18.8, 19.1, 23.1, 26.7, 27.6, 29.2, 34.8, 35.6, 36.9, 37.8, 38.0, 38.0, 38.9, 40.1, 43.8, 44.0, 48.1, 48.7, 48.7, 50.5, 65.7, 67.0, 72.9, 77.0, 211.3.

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