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Synthesis of Two Marine Natural Products: the Aglycones of Pavoninin-1 and 2#

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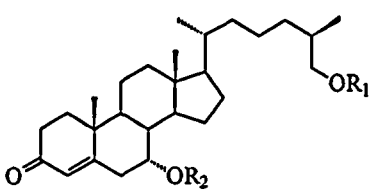
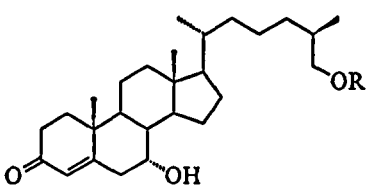
Abstract: *The first syntheses of the aglycones of pavoninin-1 and 2 have been accomplished via the epoxide 12.* © 1997 Elsevier Science Ltd.

Introduction

Pavoninin-1 and 2, steroid N-acetylglucosaminides isolated from the defense secretion of the sole *Pardachirus pavoninus*, exhibit potent bioactivity such as an ichthyotoxins, hemolytes, and shark-repellants.[1] The structures of the aglycones of pavoninin-1 and 2 reveal that these molecule have 7α and (25*R*)-26-hydroxyl groups and a cholest-4-en-3-one system(Fig.1). 7α,26-Dihydroxycholest-4-en-3-one **2** and 26-hydroxycholest-4-en-3-one have been shown to be potent inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.[2] Inhibition of HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis by some chloest-4-en-3-one analogs, is a valuable strategy in the therapeutic treatment of atherosclerosis.





Pavoninin-1: R_1 = Ac, R_2 = β-glcNAc
Pavoninin-2: R_1 = H, R_2 = β-glcNAc

1: R = Ac
2: R = H

β-glcNAc =

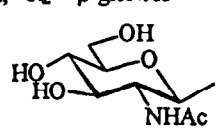


Figure 1.

#Dedicated to professor Sang Chul Shim, on the occasion of his 60th birthday.

Although compound **2** has been already synthesized by oxidation of cholest-5-ene-3β,7α,26-triol with cholesterol oxidase,[3] large quantities of this compound are needed for biological evaluation. Herein we describe the first chemical synthesis of 7α,26-dihydroxycholest-4-en-3-one **2**, *i.e.* an aglycone of payoninin-2, and 7α,26-dihydroxycholest-4-en-3-one 26-acetate **1**, an aglycone of pavoninin-1.

Results and Discussion

We were interested in the functionality shown by compounds **1** and **2**; in each the side chain has a (25*R*)-26-hydroxyl group, the A ring has an enone and the B ring has a 7α-hydroxyl group. Thus (25*R*)-26-hydroxycholesterol **3**, having suitably presented or modified functional groups, was chosen as a starting material, for the present synthesis. The synthesis of (25*R*)-26-hydroxycholesterol via the Clemmensen reduction of diosgenin and the selective protection of the 3β,26-hydroxyl groups of the resulting cholest-5-ene-3β,16β,26-triol, followed by removal of the 16-hydroxyl group, has been described previously.[4] This in turn may be transformed to our target molecule via selective protection of the 26-hydroxyl group followed by introduction of the required functionality in the A and B rings.

The selective protection can be achieved by reacting **3** with one-and-half molar excess of *tert*-butyldimethylsilyl chloride in dichloromethane at room temperature for 6 h. Usual work-ups gave products as a mixture of monosilylated compound **5** (4%) and **6** (70%) along with disilylated derivative **4** (5%). Replacing *tert*-butyldimethylsilyl chloride with *tert*-butyldiphenylsilyl chloride under the same reaction conditions save a bit longer reaction time would not affect the product distribution. It is worth noting that the desilylation of **4** can be affected to give the monosilylated products **5** (15%) and **6** (31%) in an acetic acid-water solvent system (v/v = 3/1) at 50°C.

Employment of Oppenauer oxidation[5] of 26-TBS ether **6** using aluminum isopropoxide and cyclohexanone in toluene yielded Δ^4-3-one **8** in very high yield (91%).

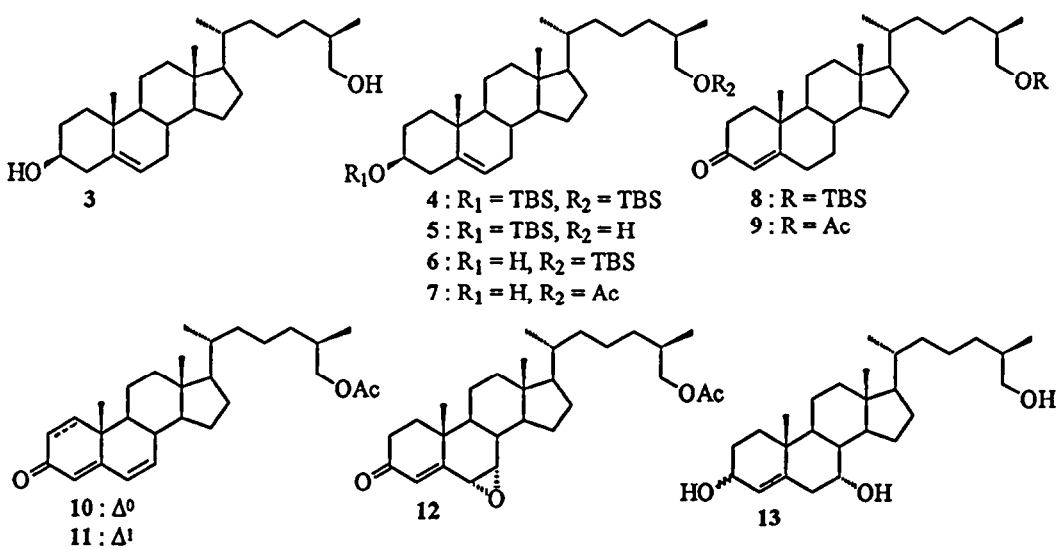


Figure 2.

Most characteristic features of **8** involve the appearance of infra red stretching band at 1676 cm^{-1} for the conjugated carbonyl group in addition to the ^{13}C NMR peak at 199.4 ppm assignable for the C-3 atom. Note that an unconjugated ketonic carbon appears at 207 ppm.

As the next step, the TBS group was replaced with the acetyl group because the TBS group would be deprotected in the subsequent dichlorodicyanobenzoquinone(DDQ) oxidation; pavoninin-1 originally had a 26-acetate group in its structure. Desilylation of 26-TBS ether **8** and acetylation were readily performed with PTSA in acetone and acetic anhydride in dry pyridine, to give compound **9** in 95% overall yield in two steps. The compound **9** can also be obtained from **5** in two steps: (i) the first step being treatment of **5** with acetic anhydride in dry pyridine followed by desilylation of TBS group with PTSA in acetone to afford **7** in 81%; and (ii) the second one being oxidation of **7** with aluminum isopropoxide yielding Δ^4-3-one **9** (76%).

Treatment of Δ^4-3-one **9** with DDQ and HCl solution in dioxane[6] resulted in $\Delta^{4,6}$-3-one **10** (76%) which carries the requisite oxygen functionality at C-7 along with $\Delta^{1,4,6}$-3-one **11** (5%). We have found that a slow addition of DDQ in this transformation is quite essential to improve the yield. Namely, fast addition via a dropping funnel in 15 min resulted in **10** (<40%) and **11** (50%) while prolonged addition for 6 h via a syringe pump gave **10** as a predominant product. The extension of a double bond was confirmed by the bathochromic shift found in **10** (λ_{max} 285 nm; log ε 4.3) as compared that of **9** (λ_{max} 242 nm; log ε 4.1). Catalytic hydrogenation of $\Delta^{1,4,6}$-3-one **11** with $Rh(PPh)_3Cl$ at atmospheric pressure of hydrogen resulted in the formation of $\Delta^{4,6}$-3-one **10** in 84% yield.

Regioselective epoxidation of **10** with *m*-chloroperoxybenzoic acid at room temperature gave the required 6α,7α-epoxide **12** in 60% yield. The epoxide obtained was expected as the α stereoisomer because the bulky reagent preferentially attacked to the sterically less hindered α direction[7] and that was confirmed by the product in the following cleavage of epoxide. Various spectroscopic data are consistent with the structure of **12**. For instance, ^1H NMR shows one vinylic proton at 6.10 ppm and the pair of epoxy protons at 3.34 and 3.44 ppm. Two olefinic carbons appear at 131.0 and 162.8 ppm and two epoxy carbons at 51.4 and 54.6 ppm on ^{13}C NMR. The UV spectrum exhibits λ_{max} at 240 nm (log ε 4.2) confirming the existence of Δ^4. The mass spectrum also reveals a corresponding molecular ion peak at m/z 456.

Cleavage of epoxide **12** by catalytic transfer hydrogenolysis with $Pd/BaSO_4$ and proton donors such as cyclohexene[6] or formic acid in methanol[8] afforded a very low yield(3%) of 7α-hydroxyl compound **1** and a complex mixture of polar products. Thus, the high yield synthesis of **2** would be desirable. 7α,26-Dihydroxycholest-4-en-3-one **2** can be prepared in a relatively high yield (71%) from the reduction of **12** with $LiAlH_4$ followed by selective MnO_2 oxidation[9]. Selective acetylation of **2** with acetic anhydride in pyridine at 10°C gave 7α,26-dihydroxycholest-4-en-3-one 26-acetate **1** in 71% yield. During this transformation to ensure the configuration of the hydroxyl group at C-7 is essential, the ^{13}C NMR chemical shift of which can be the determining factor. The δ of the synthetic compound **2**, 68.5 ppm, in $CDCl_3$ is like that in 7α-hydroxycholestane(68.2 ppm), and unlike that in 7β-hydroxycholestane(75.2 ppm).[10] ^1H NMR is also quite informative to reveals H-4 and H-7β at 5.80 and 3.97 ppm(J = 2.1), respectively. These are in good agreement with the reported value for the corresponding compounds.[1a]

In summary, 7α,26-dihydroxycholest-4-en-3-one **2** and 26-acetate **1** have been prepared from (25*R*)-26-hydroxycholesterol. The synthetic methodology developed for these interesting marine natural products is being utilized in the preparation of analogs. We are currently evaluating the biological activities of the aglycones of pavoninin-1 and 2.

Experimental Section

General. Melting points were measured using a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a Matton GL-6030E spectrophotometer. UV spectra were determined in methanol on a Shimadzu UV-2100 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on either Bruker AM-300 or JEOL GSX-500 instruments; unless otherwise stated all NMR were performed in $CDCl_3$ solution. The chemical shifts of ^1H NMR spectra are given in ppm downfield from tetramethylsilane and ^{13}C NMR spectra were referenced to $CDCl_3$ at 77.0 ppm. Coupling constant J values are given in Hz. ^1H and ^{13}C NMR assignments were made from DEPT, COSY, HETCOR, and comparison with spectra of similar sterols.[1,11] Low resolution mass spectra(MS) were recorded on a Shimadzu QP-1000 spectrometer with electron energy of 20 or 70eV and direct sample introduction. High resolution MS were measured on a JEOL JMS-DX 303 spectrometer. Microanalyses were performed using a Carlo Erba 1106 elemental analyser. TLC analyses were carried out on precoated 0.2 mm HPTLC silica gel 60 plates (E. Merck, Darmstadt); substances were visualized by spraying with 5% ammonium molybdate in 10% H_2SO_4 followed by heating. Medium pressure liquid chromatography (MPLC) was performed by using EM Lobar Silica Gel 60 prepacked columns (40-63μm; A, B, C type; E. Merck, Darmstadt) equipped with a Fluid Metering Inc. lab pump. For routine column chromatography, E. Merck silica gel (70-230 mesh) was used as adsorbent. Solvents were distilled before use and were dried, as necessary, by literature procedures. Solutions were dried over anhydrous sodium sulfate. (25*R*)-26-Hydroxycholesterol **3** was prepared previously.[4]

Protection of (25*R*)-26-Hydroxycholesterol with *tert*-butyldimethylsilyl chloride. To a solution of (25*R*)-26-hydroxycholesterol (1.52 g, 3.79 mmol) in dry $CHCl_3$ (50 mL) were added 4-dimethylaminopyridine (20 mg, 0.16 mmol), triethylamine (3 mL) and then dropwise *tert*-butyldimethylsilyl chloride (858 mg, 5.68 mmol) in $CHCl_3$ (3 mL) in an ice bath for 3 h. The ice bath was removed and stirred at room temperature for 6 h, and then quenched with water. The organic layer was separated, washed successively with water, saturated aqueous sodium hydrogen carbonate solution, and brine, dried, and evaporated. The residue was subjected to MPLC eluted with 10% ethyl acetate in hexane to give successively compound **4** (120 mg, 5%), **5** (82 mg, 4%), **6** (1.36 g, 70%) and **3** (286 mg, 19%).

(25*R*)-3β,26-Bis(*tert*-butyldimethylsilyloxy)cholest-5-ene-3β,26-diol (4). mp 95°C (CH_2Cl_2-MeOH); TLC R_f 0.43 (3% EtOAc in hexane), 0.77 (3% Et_2O in benzene); IR ν_{max}: 2934, 2857, 1082, 837 cm^{-1}; ^1H NMR δ: 0.034 (6H, s, $Si(CH_3)_2$), 0.054(6H, s, $Si(CH_3)_2$), 0.674 (3H, s, 18-CH_3), 0.858 (3H, d, J = 6.7 Hz, 27-CH_3), 0.888 (9H, s, $SiC(CH_3)_3$), 0.894 (9H, s, $SiC(CH_3)_3$), 0.910 (3H, d, J = 6.6 Hz, 21-CH_3), 0.999 (3H, s, 19-CH_3), 3.35 (1H, dd, J = 9.8, 6.6 Hz, 26-H_a), 3.43 (1H, dd, J = 9.8, 5.9 Hz, 26-H_b), 3.46 (1H, m, 3α-H), 5.3 (1H, d, J = 5.1 Hz, 6-H); ^{13}C NMR δ: -4.6 and -5.3(Si-CH_3), 11.9, 16.7, 18.2(Si-$C(CH_3)_3$), 18.7, 19.4, 21.1, 23.4, 24.3, 25.9(Si-C(CH_3)$_3$), 28.3, 32.0, 32.0, 32.1, 35.7, 33.6, 35.8, 36.2, 36.6, 37.4, 39.9, 42.4, 42.9, 50.3, 56.2, 56.9, 68.6, 72.7, 121.2, 141.6; m/z 616(3%, M-CH_3), 574 (69, M-C_4H_9), 499 (4, M-TBSiOH), 484 (3, M-TBSiOH-CH_3), 442 (25, M-TBSiOH-C_4H_9), 367 (3, M-2TBSiOH), 256 (2, M-SC-TBSiOH).

(25*R*)-3β-(*tert*-Butyldimethylsilyloxy)cholest-5-ene-3β,26-diol (5). mp 173-175°C (CH_2Cl_2-MeOH); TLC R_f 0.45 (1:4 EtOAc/hexane), 0.48 (1:4 Et_2O/benzene); IR ν_{max}: 3380, 2936, 2859, 1466, 1375, 1252, 1098, 837 cm^{-1}; ^1H NMR δ: 0.035 (6H, s, $Si(CH_3)_2$), 0.656 (3H, s, 18-CH_3), 0.870 (9H, s, $SiC(CH_3)_3$), 0.873 (3H, d, J = 6.7 Hz, 27-CH_3), 0.895(3H, d, J = 6.7 Hz, 21-CH_3), 0.980 (3H, s, 19-CH_3), 3.39 (1H, dd, J = 10.3, 6.6 Hz, 26-H_a), 3.48 (1H, dd, J = 10.3, 5.8 Hz, 26-H_b), 3.45 (1H, m, 3α-H), 5.3 (1H, d, J = 5.2 Hz, 6-

H); ^{13}C NMR δ: -4.6(Si-*C*H$_3$), 11.9, 16.5, 18.2(Si-*C*(CH$_3$)$_3$), 18.7, 19.4, 21.1, 23.4, 24.3, 25.9(Si-C(*C*H$_3$)$_3$), 28.3, 32.0, 32.0, 32.1, 33.6, 35.7, 35.8, 36.2, 36.6, 37.4, 39.9, 42.4, 42.9, 50.3, 56.2, 56.8, 68.5, 72.7, 121.1, 141.6; m/z 459 (100%, M-C$_4$H$_9$), 441 (8, M-C$_4$H$_9$-H$_2$O), 385 (7), 367 (16), 255 (7, M-SC-TBSiOH); HRMS: Calcd for (M-C$_4$H$_9$) $C_{29}H_{51}O_2Si$ 459.3658: Found: 459.3650.

(25*R*)-26-(*tert*-Butyldimethylsilyloxy)cholest-5-ene-3β,26-diol (6). mp 91-92°C (CH_2Cl_2-MeOH); TLC R_f 0.32 (1:4 EtOAc/hexane), 0.34 (1:4 Et_2O/benzene); IR ν_{max}: 3391, 2938, 2899, 2859, 1466, 1254, 1101, 1057, 843 cm^{-1}; ^{1}H NMR δ: 0.117 (6H, s, Si(CH$_3$)$_2$), 0.656 (3H, s, 18-CH$_3$), 0.835 (3H, d, *J* = 6.8 Hz, 27-CH$_3$), 0.871 (9H, s, SiC(CH$_3$)$_3$), 0.892 (3H, d, *J* = 6.8 Hz, 21-CH$_3$), 0.985 (3H, s, 19-CH$_3$), 3.33 (1H, dd, *J* = 10.3, 6.7 Hz, 26-H$_a$), 3.41 (1H, dd, *J* = 10.3, 5.9 Hz, 26-H$_b$), 3.50 (1H, m, 3α-H), 5.32 (1H, d, *J* = 5.1 Hz, 6-H); ^{13}C NMR δ: -5.4(Si-*C*H$_3$), 11.9, 16.7, 18.3(Si-*C*(CH$_3$)$_3$), 18.7, 19.4, 21.1, 23.4, 24.3, 25.9(Si-C(*C*H$_3$)$_3$), 28.2, 31.7, 31.9, 31.9, 33.6, 35.7, 35.7, 36.2, 36.5, 37.3, 39.8, 42.3, 42.3, 50.2, 56.2, 56.8, 68.5, 71.8, 121.7, 140.8.; m/z 498(3%, M-H$_2$O), 459 (5, M-C$_4$H$_9$), 441 (15, M-C$_4$H$_9$-H$_2$O), 367 (3), 255 (4, M-SC-H$_2$O); HRMS: Calcd for (M-C$_4$H$_9$) $C_{29}H_{51}O_2Si$ 459.3658: Found: 459.3653.

(25*R*)-Cholest-5-ene-3β,26-diol 26-acetate (7). To a solution of **5** (480 mg, 0.93 mmol) in dry pyridine (20 mL) was added acetic anhydride (1.5 mL) then stirred at room temperature for 2 h. After the reaction was completed, water was added to the reaction mixture, and extracted twice with ethyl acetate. The combined organic layer was washed with 10% HCl solution, water, and saturated aqueous sodium hydrogen carbonate solution, dried and concentrated. To the crude product, dissolved in acetone (20 mL) was added PTSA (530 mg, 2.79 mmol) at room temperature then stirred for 2 h. The reaction mixture was diluted with water and extracted twice with ethyl acetate. The combined organic layers were washed with water, and saturated aqueous sodium hydrogen carbonate solution, dried, and evaporated. The crude product was purified by silica gel column chromatography eluted with 20% ethyl acetate in hexane to give **7** (335 mg, 81%). mp 78-79°C (CH_2Cl_2-hexane); TLC R_f 0.50 (1:2 EtOAc/hexane), 0.47 (1:2 Et_2O/benzene); IR ν_{max}: 3392, 3315, 2937, 2902, 2863, 1740, 1466, 1374, 1254, 1061, 1034 cm^{-1}; ^{1}H NMR δ: 0.683 (3H, s, 18-CH$_3$), 0.918 (6H, d, *J* = 6.8 Hz, 21, 27-CH$_3$), 1.009 (3H, s, 19-CH$_3$), 2.05 (3H, s, CH$_3$CO), 3.51 (1H, m, 3α-H), 3.84 (1H, dd, *J* = 10.6, 6.8 Hz, 26-H$_a$), 3.95 (1H, dd, *J* = 10.6, 6.0 Hz, 26-H$_b$), 5.34 (1H, d, *J* = 5.3 Hz, 6-H); ^{13}C NMR δ: 11.8, 16.8, 19.4, 19.7, 20.9, 21.1, 23.3, 24.3, 28.2, 31.7, 31.9, 31.9, 32.5, 33.8, 35.7, 36.1, 36.5, 37.3, 39.8, 42.3, 42.3, 50.2, 56.2, 56.8, 69.6, 71.7, 121.6, 140.8, 171.2; m/z 444 (7%, M$^+$), 426 (26, M-H$_2$O), 411 (10, M-H$_2$O-CH$_3$), 384 (5, M-HOAc), 273 (5, M-SC), 255 (10, M-SC-H$_2$O); HRMS: Calcd for $C_{29}H_{48}O_3$ 444.3603: Found: 444.3602.

(25*R*)-26-(*tert*-Butyldimethylsilyloxy)cholest-4-en-3-one (8). A solution of **6** (430 mg, 0.83 mmol) and cyclohexanone (3 mL) in toluene (50 mL) was heated with Dean Stark column to remove water. Al(*i*-PrO)$_3$ (340 mg, 1.66 mmol) was added to the mixture then refluxed with exclusion of moisture for 4 h. The resulting mixture was cooled, added saturated aqueous sodium hydrogen carbonate solution, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel column eluted with 10% ethyl acetate in hexane to give **8** (386 mg, 91%) as an oil, TLC R_f 0.75 (1:2 EtOAc/hexane), 0.72 (1:2 Et_2O/benzene); IR ν_{max}: 2934, 2857, 1676 (α,β-unsaturated C=O), 1464, 1251, 1096, 837 cm^{-1}; ^{1}H NMR δ: 0.038 (6H, s, Si(CH$_3$)$_2$), 0.724 (3H, s, 18-CH$_3$), 0.861 (3H, d, *J* = 6.7 Hz, 27-CH$_3$), 0.896 (9H, s, SiC(CH$_3$)$_3$), 0.926 (3H, d, *J* = 6.5 Hz, 21-CH$_3$), 1.165 (3H, s, 19-CH$_3$), 3.36 (1H, dd, *J* = 9.7, 6.5 Hz, 26-H$_a$), 3.43 (1H, dd, *J* = 9.7, 5.9 Hz, 26-H$_b$), 6.17 (1H, s, 4-H); ^{13}C NMR δ: -5.4(Si-*C*H$_3$), 11.9, 16.6, 17.5, 18.3(Si-*C*(CH$_3$)$_3$), 18.6, 20.9, 23.3, 23.9, 25.9(Si-C(*C*H$_3$)$_3$), 28.0, 33.5, 33.5, 33.9, 34.2, 35.6, 35.7,

35.7, 36.1, 39.2, 39.8, 42.5, 46.8, 51.0, 56.0, 56.6, 68.5, 125.4, 161.0, 199.4; m/z 457 (89%, M-C_4H_9); HRMS: Calcd for (M-C_4H_9) $C_{29}H_{49}O_2Si$ 457.3502: Found: 457.3481.

(25*R*)-26-Hydroxycholest-4-en-3-one 26-acetate (9). To a solution of **8** (487 mg, 0.95 mmol) in acetone (30 mL) was added PTSA (541 mg, 2.84 mmol) at room temperature then stirred for 2 h. The reaction mixture was diluted with water and extracted twice with ethyl acetate. The combined organic layers were washed with water, dried, and evaporated. To the crude product, dissolved in dry pyridine (20 mL), was added acetic anhydride (1.5 mL) then stirred at room temperature for 2 h. After the reaction was completed, water was added to the reaction mixture, and extracted twice with ethyl acetate. The combined organic layer was washed with 10% HCl solution, water, and saturated aqueous sodium hydrogen carbonate solution, dried and concentrated. The crude product was purified by silica gel column chromatography eluted with 20% ethyl acetate in hexane to give **9** (398 mg, 95%) as a viscous oil. TLC R_f 0.62 (1:2 EtOAc/hexane), 0.62 (1:2 Et_2O/benzene); IR ν_{max}: 2941, 2870, 1740, 1676 (α,β-unsaturated C=O), 1616, 1464, 1375, 1235, 1036 cm^{-1}; UV λ_{max}: 242 nm (log ε 4.1); ^1H NMR δ: 0.715 (3H, s, 18-CH_3), 0.967 (6H, d, J = 6.6 Hz, 21, 27-CH_3), 1.183 (3H, s, 19-CH_3), 2.04 (3H, s, CH_3CO), 3.84 (1H, dd, J = 10.7, 6.9 Hz, 26-H_a), 3.95 (1H, dd, J = 10.7, 6.0 Hz, 26-H_b), 5.71 (1H, s, 4-H); ^{13}C NMR δ: 11.8, 16.7, 17.3, 18.5, 20.8, 20.9, 23.1, 24.0, 28.0, 31.9, 32.4, 32.8, 33.6, 33.8, 35.5, 35.5, 35.6, 35.9, 38.5, 39.5, 42.3, 53.7, 55.8, 56.0, 69.4, 123.6, 171.0, 171.2, 199.2; m/z 442 (55%, M^+), 427 (5, M-CH_3), 382 (9, M-HOAc), 271 (12, M-SC); HRMS: Calcd for $C_{29}H_{46}O_3$ 442.3447: Found: 442.3445.

(25*R*)-26-Hydroxycholest-4,6-dien-3-one 26-acetate (10). To a solution of **9** (200 mg, 0.45 mmol) in dioxane (15 mL) and concentrated HCl (0.1 mL) was added dropwise DDQ (123 mg, 1.54 mmol) in dioxane (3 mL) at room temperature for 6 h with stirring. The reaction was quenched with saturated aqueous sodium hydrogen carbonate solution, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated. The crude product was purified by silica gel chromatography eluted with 20% ethyl acetate in hexane to give diene **10** (151 mg, 76%) as a viscous oil. TLC R_f 0.60 (1:2 EtOAc/hexane), 0.61 (1:2 Et_2O/benzene); IR ν_{max}: 2941, 2867, 1740, 1665 (α,β-unsaturated C=O), 1617, 1466, 1376, 1236, 1035, 877 cm^{-1}; UV λ_{max}: 285 nm (log ε 4.3); ^1H NMR($CDCl_3$+DMSO-d_6) δ: 0.765 (3H, s, 18-CH_3), 0.920 (3H, d, J = 6.6 Hz, 27-CH_3), 0.930 (3H, d, J = 6.5 Hz, 21-CH_3), 1.117 (3H, s, 19-CH_3), 2.04 (3H, s, CH_3CO), 3.83 (1H, dd, J = 10.7, 6.9 Hz, 26-H_a), 3.93 (1H, dd, J = 10.7, 6.0 Hz, 26-H_b), 5.63 (1H, bs, 4-H), 6.09 (1H, dd, J = 9.8, 2.3 Hz, 6-H), 6.16 (1H, d, J = 9.8 Hz, 7-H); ^{13}C NMR($CDCl_3$+DMSO-d_6) δ: 11.3, 15.6, 16.1, 18.0, 20.0, 20.3, 22.6, 23.0, 27.5, 31.8, 33.3, 33.3, 35.3, 35.3, 35.4, 37.1, 38.9, 39.4, 42.8, 50.1, 52.8, 55.4, 68.8, 122.7, 127.9, 141.0, 163.5, 170.4, 190.7; m/z 441 (100%, M^++1), 440 (80, M^+), 426 (7, M+1-CH_3), 380 (5, M-HOAc), 269 (15, M-SC); HRMS: Calcd for $C_{29}H_{44}O_3$ 440.3290: Found: 440.3301, and triene **11** (10 mg, 5%) as a viscous oil. TLC R_f 0.55 (1:2 EtOAc/hexane), 0.56 (1:2 Et_2O/benzene); IR ν_{max}: 2938, 2871, 1740, 1655 (α,β-unsaturated C=O), 1604, 1459, 1375, 1285, 1237, 1035, 890 cm^{-1}; UV λ_{max}: 301 (log ε 4.4), 257 (log ε 4.3), 224 nm (log ε 4.4); ^1H NMR δ: 0.738 (3H, s, 18-CH_3), 0.920 (6H, d, J = 7.0 Hz, 21, 27-CH_3), 1.195 (3H, s, 19-CH_3), 2.05 (3H, s, CH_3CO), 3.84 (1H, dd, J = 10.7, 6.8 Hz, 26-H_a), 3.95 (1H, dd, J = 10.7, 5.9 Hz, 26-H_b), 6.00 (1H, bs, 4-H), 6.04 (1H, dd, J = 9.9, 1.8 Hz, 6-H), 6.22 (1H, d, J = 9.9 Hz, 2-H), 6.24 (1H, dd, J = 9.9, 1.8 Hz, 7-H), 7.06 (1H, d, J = 9.9 Hz, 1-H); ^{13}C NMR δ: 11.9, 16.7, 18.5, 20.7, 20.9, 21.8, 23.3, 23.6, 28.1, 32.5, 33.7, 35.6, 35.9, 38.2, 39.5, 41.2, 43.0, 48.3, 53.6, 55.9, 69.5, 123.6, 127.5, 128.1, 138.7, 153.0, 162.7, 171.2, 186.3; m/z 439 (100%, M^++1), 438 (85, M^+), 423 (27, M-CH_3), 363 (22, M-HOAc-CH_3), 267 (83, M-SC); HRMS: Calcd for $C_{29}H_{42}O_3$ 438.3134: Found: 438.3137.

Selective hydrogenation of 26-hydoxycholest-1,4,6-trien-3-one 26-acetate 11 to 10. In advance, the solution of **11** (260 mg, 0.59 mmol) in ethanol (30 mL) was bubbled with H_2 gas for 10 min, and then 0.03 equiv of $RhCl(PPh_3)_3$ (16 mg) was added and refluxed under 1 atm of H_2 for 20 h. The solvent was removed under reduced pressure, and added ethyl ether (50 mL). The catalyst was removed by filtration on a short silica gel column. The evaporated residue was chromatographed on silica gel column eluted with 20% ethyl acetate in hexane to give diene **10** (220 mg, 84%) and triene **11** (20 mg, 8%).

(25*R*)-26-Hydroxycholest-4-en-6α,7α-epoxy-3-one 26-acetate (12). To a solution of **10** (105 mg, 0.24 mmol) in CH_2Cl_2 (15 mL) was added dropwise a solution of *m*-chloroperoxybenzoic acid (134 mg, 0.43 mmol) in CH_2Cl_2 (5 mL) at 0°C for 15 min. The mixture was removed from the ice bath and stirred at room temperature for 5 days. To the resulting mixture was added cooled saturated aqueous sodium hydrogen carbonate solution and extracted with CH_2Cl_2. The organic layer was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel column eluted with 20% ethyl acetate in hexane to give **12** (65 mg, 60%). mp 63-64°C (CH_2Cl_2-hexane); TLC R_f 0.50 (1:2 EtOAc/hexane), 0.56 (1:2 Et_2O/benzene); IR ν_{max}: 2944, 2867, 1740, 1679 (α,β-unsaturated C=O), 1459, 1378, 1239, 1038 cm^{-1}; UV λ_{max}: 240 nm (log ε 4.2); ^1H NMR δ: 0.737 (3H, s, 18-CH_3), 0.920 (6H, d, J = 6.7 Hz, 21, 27-CH_3), 1.093 (3H, s, 19-CH_3), 2.05 (3H, s, CH_3CO), 3.34 (1H, d, J = 3.6 Hz, 7-H), 3.44 (1H, d, J = 3.6 Hz, 6-H), 3.84 (1H, dd, J = 10.7, 6.9 Hz, 26-H_a), 3.95 (1H, dd, J = 10.7, 6.0 Hz, 26-H_b), 6.10 (1H, s, 4-H); ^{13}C NMR δ: 11.8, 16.7, 17.2, 18.6, 19.9, 20.9, 23.3, 23.6, 28.3, 32.5, 33.7, 33.9, 34.1, 34.6, 35.6, 35.7, 36.0, 39.3, 40.6, 43.0, 51.4, 52.5, 54.6, 55.8, 69.5, 131.0, 162.8, 171.2, 198.2; m/z 456 (62%, M^+), 441 (100, M-CH_3), 285 (43, M-SC). Anal. Calcd for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71: Found: C, 76.42; H, 9.49.

(25*R*)-7α,26-Dihydroxycholest-4-en-3-one (2). To a solution of **12** (10 mg, 0.02 mmol) in dry THF (5 mL) was added $LiAlH_4$ (2.5 mg, 0.06 mmol) in an ice bath. The mixture was stirred at room temperature under nitrogen for 2 h. The excess reagent was destroyed by the careful addition of ethyl acetate. The reaction product was treated with 5% HCl solution and extracted with ethyl acetate. The extract was washed in turn with water, saturated aqueous sodium hydrogen carbonate solution, and brine, dried, and evaporated. The crude product **13**, dissolved in dry CH_2Cl_2 (5 mL), was stirred with 100 mg of manganese dioxide at room temperature for 30 min. The reaction mixture was filtered on celite to remove inorganic material. The filtrate was concentrated and the residue was chromatographed on silica gel column eluted with 50% ethyl acetate in hexane to give **2** (6.5 mg, 71%). mp 168-169°C (CH_2Cl_2-hexane); TLC R_f 0.51 (EtOAc), 0.26 (Et_2O); IR ν_{max}: 3415, 2937, 2871, 1659, 1459, 1378, 1034 cm^{-1}; UV λ_{max}: 242 nm (log ε 4.2); ^1H NMR δ: 0.716 (3H, s, 18-CH_3), 0.915 (3H, d, J = 6.9 Hz, 27-CH_3), 0.922 (3H, d, J = 6.3 Hz, 21-CH_3), 1.193 (3H, s, 19-CH_3), 3.42 (1H, dd, J = 10.4, 6.5 Hz, 26-H_a), 3.50 (1H, dd, J = 10.4, 5.9 Hz, 26-H_b), 3.97 (1H, d, J = 2.0 Hz, 7β-H), 5.80 (1H, d, J = 1.5 Hz, 4-H); ^{13}C NMR(CD_3OD) δ: 12.2, 17.0, 17.4, 19.2, 22.0, 24.4, 24.5, 29.3, 34.8, 34.8, 36.5, 36.9, 37.1, 37.3, 39.8, 40.7, 41.0, 42.2, 43.5, 46.4, 51.7, 57.5, 68.6, 69.2, 126.7, 172.8, 201.9; m/z 416 (27%, M^+), 401 (22, M-CH_3), 398 (22, M-H_2O), 360 (37), 269 (22, M-H_2O-SC); HRMS: Calcd for $C_{27}H_{44}O_3$ 416.3290: Found: 416.3284.

(25*R*)-7α,26-Dihydroxycholest-4-en-3-one 26-acetate (1). To a solution of **2** (14.5 mg, 0.04 mmol) in dry pyridine (2 mL) was added dropwise acetic anhydride (0.1 mL) diluted with pyridine (1 mL) in an ice bath for 1 h and then stirred at 10°C for additional 3 h. To the mixture was added methanol (0.1 mL) and extracted twice with ethyl acetate (20 mL). The combined organic layers were washed with 10% HCl solution, water, and saturated aqueous sodium hydrogen carbonate solution, dried, and concentrated. The residue was

chromatographed on silica gel column eluted with 30% ethyl acetate in hexane to give **1** (11.3 mg, 71%). mp 128-129°C (Et_2O-hexane); TLC R_f 0.42 (1:1 EtOAc/hexane), 0.24 (1:1 Et_2O/benzene); IR ν_{max}: 3423, 2933, 2867, 1736, 1667, 1378, 1239, 1042 cm^{-1}; UV λ_{max}: 242 nm (log ε 4.2); ^1H NMR δ: 0.718 (3H, s, 18-CH_3), 0.919 (6H, d, J = 6.6 Hz, 21, 27-CH_3), 1.95 (3H, s, 19-CH_3), 2.06 (3H, s, CH_3CO), 3.84 (1H, dd, J = 10.8, 6.9 Hz, 26-H_a), 3.95 (1H, dd, J = 10.8, 6.0 Hz, 26-H_b), 3.97 (1H, d, J = 2.1 Hz, 7β-H), 5.81 (1H, d, J = 1.8 Hz, 4-H); ^{13}C NMR($CDCl_3$) δ: 11.8, 16.8, 17.0, 18.6, 20.8, 21.0, 23.2, 23.6, 28.2, 32.5, 33.7, 34.0, 35.4, 35.7, 35.9, 38.5, 39.2, 39.8, 40.9, 42.4, 45.2, 50.4, 56.0, 68.5, 69.6, 126.8, 167.7, 171.3, 198.8; m/z 458 (36%, M^+), 440 (84, M-H_2O), 425 (55, M-H_2O-CH_3), 402 (100), 398 (27, M-HOAc), 269 (26, M-H_2O-SC); HRMS: Calcd for $C_{29}H_{46}O_4$ 458.3396: Found: 458.3389.

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