New Polyhydroxylated Cholestane Glycosides from the Bulbs of *Ornithogalum saundersiae*

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Fresh bulbs of *Ornithogalum saundersiae* were found to contain five new polyhydroxylated cholestane glycosides. Their structures were determined to be (22*S*)-cholest-5-ene-3β,11β,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside (1) and its acetyl derivatives (2 and 3), and (22*S*)-cholesta-5,24-diene-3β,11β,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside (4) and its acetyl derivative (5) using spectroscopic analysis and chemical correlations. Determination of the absolute configuration at the C-22 hydroxyl position of 1 was achieved by the application of the advanced Mosher's method to the aglycon (1a) of 1, and those of 2—5 by correlating them to 1. The cholestane glycosides with an acetyl group at the C-3 hydroxyl position of the rhamnose showed potent inhibitory activity on cyclic adenosine monophosphate (AMP) phosphodiesterase.

Keywords *Ornithogalum saundersiae*; Liliaceae; polyhydroxylated cholestane glycoside; advanced Mosher's method; cyclic AMP phosphodiesterase; inhibitory activity

Some *Ornithogalum* plants are known to contain cardenolide glycosides.[1] Our previous examinations have shown the bulbs of *Ornithogalum thyrsoides*[2] and *O. saundersiae*[3] to be devoid of cardenolide glycosides and to contain cholestane glycosides as characteristic constituents. Further analysis of the 1-butanol-soluble phase of the methanolic extract of *O. saundersiae* bulbs led to the isolation of five new polyhydroxylated cholestane glycosides. This paper deals with the structural elucidation of the glycosides based on spectroscopic analysis and chemical correlations, and their inhibitory activity on cyclic adenosine monophosphate (AMP) phosphodiesterase.

The methanolic extract of the bulbs was further fractionated by silica gel column chromatography and reversed-phase high-performance liquid chromatography (HPLC) to give new compounds **1**—**5** in addition to the glycosides reported earlier.[3]

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D$ −39° (methanol). The molecular formula, $C_{33}H_{56}O_8$, was suggested by the negative-ion fast-atom-bombardment mass spectrum (FAB-MS) (*m/z* 579 $[M-H]^-$) and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra. The infrared (IR) spectrum featured a strong absorption band at 3420 cm^{-1} due to hydroxyl groups. On acid hydrolysis of **1** with 1 N hydrochloric acid in dioxane–H_2O (1 : 1), **1** liberated L-rhamnose, which was identified by direct thin-layer chromatography (TLC) comparison with an authentic sample and its specific rotation, and an aglycon (**1a**), $C_{27}H_{46}O_4$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1a** showed signals for two tertiary methyl protons at δ 1.41 and 1.25 (each s), three secondary methyl protons at δ 1.21 (d, J=7.0 Hz), 0.89 (3H, d, J=6.6 Hz) and 0.88 (d, J=6.6 Hz), and an olefinic proton at δ 5.50 (br d, J=5.3 Hz). A total of 27 carbon signals observed in the ^{13}C-NMR spectrum of **1a** were separated into Me × 5, CH_2 × 8, CH × 11 and C × 3 with the help of distortionless enhancement by polarization transfer (DEPT) spectra. The ^{13}C signals at δ 142.9 (C) and 121.0 (CH), and 75.1 (CH), 71.8 (CH), 71.7 (CH) and 68.2 (CH) were apparently assigned to a pair of olefinic carbons, and the carbons bearing hydroxyl groups. The above results were indicative of the fundamental skeleton of **1a** being a cholestene derivative with four hydroxyl groups. The presence of 3β-hydroxy-5-ene groups was readily revealed by the comparison of the ^{1}H- and ^{13}C-NMR spectra

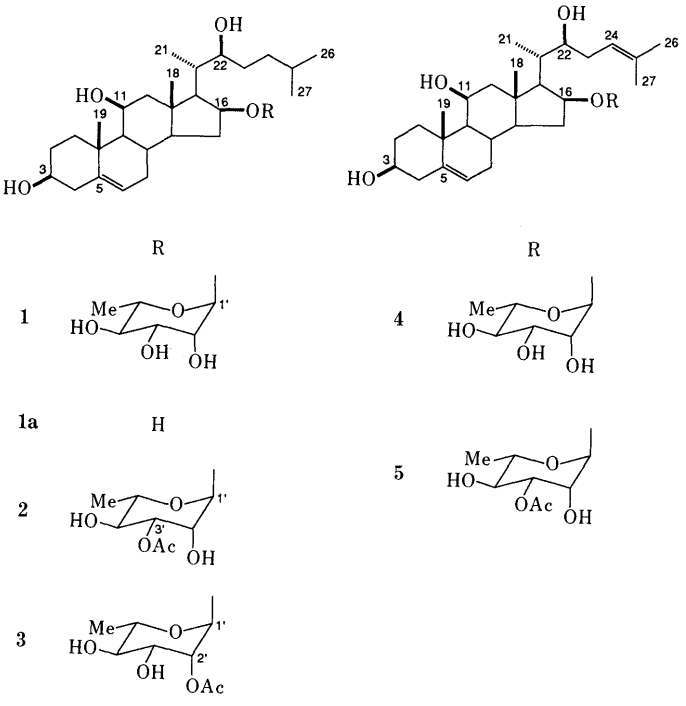

Chart 1

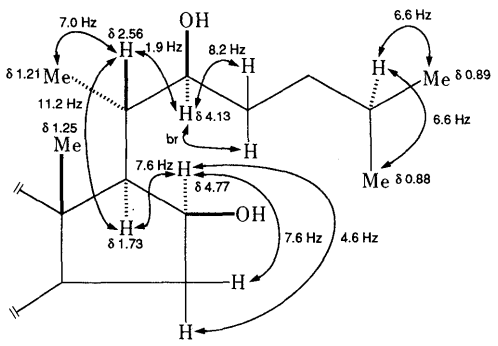

Fig. 1. ^{1}H-NMR Chemical Shifts and Spin-Coupling Constants of **1a** in Pyridine-d_5

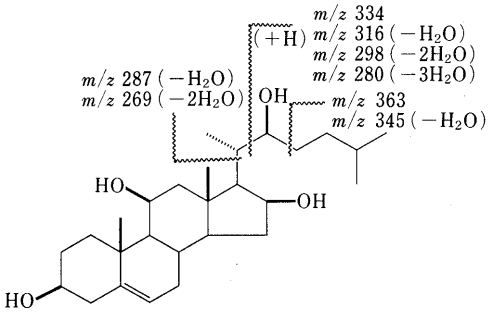

Fig. 2

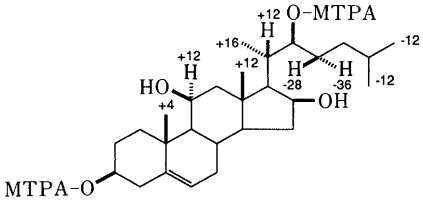

Fig. 3. Chemical Shift Differences between **1a** (*S*)-MTPA Ester (**1c**) and **1a** (*R*)-MTPA Ester (**1b**)

$\Delta\delta$ (Hz) $= \delta_{(S)\text{-MTPA}} - \delta_{(R)\text{-MTPA}}$

between **1a** and cholesterol. By tracing out the ^{1}H–^{1}H coupling network from the three-proton doublet signal at δ 1.21 assignable to the H-21 methyl proton through double resonance experiments, the existence of the C-16 and C-22 hydroxyl groups was indicated (Fig. 1), and this was further supported by agreement of the ^{13}C signals arising from the D-ring and side-chain carbons of **1a** with those of (22*S*)-cholest-5-ene-3β,16β,22-triol.[4] The fragment ion peaks observed in the electron impact mass spectrum (EI-MS) of **1a** were consistent with the presence of the C-22 hydroxyl group (Fig. 2). The remaining hydroxyl group was concluded to be located at the C-11 position because in the ^{13}C-NMR spectrum of **1a**, the signal due to the C-11 methylene carbon, which was usually observed at the most upfield position (δ 22—20) among the methylene carbons of the cholestane skeleton, was displaced by the signal due to a hydroxymethine carbon (δ 68.2), accompanied by downfield shifts of the signals due to the C-9 and C-12 carbons by 6.7 and 11.9 ppm, respectively, as compared with those of (22*S*)-cholest-5-ene-3β,16β,22-triol. The signal due to the H-18 methyl proton in the ^{1}H-NMR spectrum of **1a** (pyridine-d_5) was extremely shifted to lower field by 0.55 ppm to resonate at δ 1.25 as compared with that of cholesterol (δ 0.70), which must have been caused by the pyridine-induced deshielding effect of the β-hydroxyl groups at the C-11 and C-16 positions. Also, the H-19 methyl proton interacted with the β-hydroxyl group at the C-11 to appear at δ 1.41, which was shifted to lower field by 0.33 ppm as compared with that of cholesterol (δ 1.08). To determine the absolute configuration of the C-22 position, **1a** was converted to 3β,22-bis-(*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) derivatives (**1b** and **1c**, respectively), to which the advanced Mosher's method was applied.[5] From the comparison of the ^{1}H-NMR signals between **1b** and **1c** assigned by double resonance experiments, the $\Delta\delta$ values [$\delta(S)-\delta(R)$] were calculated as shown in Fig. 3; the H-23 methylene proton, and H-26 and H-27 methyl proton signals of **1b** appeared at lower fields than those of **1c**, and the H-20 and H-11 methine proton, as well as the H-21, H-18 and H-19 methyl proton signals of **1b** appeared at higher fields than those of **1c**. The MTPA group linked to the C-3β hydroxyl group had no influence on the H-18 and H-19 methyl, and side-chain protons.[5] Thus, the structure of **1a**, including the absolute configuration of the C-22 position, was confirmed to be (22*S*)-cholest-5-ene-3β,11β,16β,22-tetrol. The ^{13}C-NMR signals arising from the L-rhamnose moiety of **1** were δ 104.9, 72.4, 72.7, 74.0, 71.0 and 18.4 (C-1′—C-6′), and the orientation of the anomeric center was shown to be α by the comparison of the shifts with those of authentic methyl α- and β-L-rhamnopyranosides.[6] The glycosylation-induced downfield shift of the ^{13}C-NMR signal was observed at the C-16 carbon signal in **1** when all ^{13}C signals of **1** were compared with those of **1a**, accounting for the rhamnose moiety linkage to the C-16 hydroxyl group. Based on the above data, the structure of **1** was established as (22*S*)-cholest-5-ene-3β,11β,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside.

The negative-ion FAB-MS of **2** exhibited an $[M-H]^-$ ion at *m/z* 621, which exceeded that of **1** by 42 mass units. The presence of an acetyl group in the molecule was shown by the IR (1720 cm^{-1}), ^{1}H-NMR [δ 1.94 (3H, s)] and ^{13}C-NMR (δ 170.7 and 21.1) spectra. When **2** was treated with 3% sodium methoxide in methanol, **2** was hydrolyzed to yield **1**. Therefore, **2** must be a monoacetate of 1. In the ^{13}C-NMR spectrum of **2**, the signal due to the rhamnose C-3 carbon was shifted to lower field by 3.6 ppm, whereas the signals due to the C-2 and C-4 to upper fields by 2.0 and 2.8 ppm, respectively, as compared with those of **1**. Furthermore, the downfield-shifted ^{1}H signal at δ 5.75 was assigned to the rhamnose H-3 proton by its multiplicity (doublet of doublets) with the *J* values, 9.6 and 2.7 Hz. Thus, the acetyl moiety was shown to be linked to the rhamnose C-3 hydroxyl position, and the structure of **2** was determined to be (22*S*)-cholest-5-ene-3β,11β,16β,22-tetrol 16-*O*-(3-*O*-acetyl-α-L-rhamnopyranoside).

Compound **3** was also a monoacetate of **1**, and the acetyl moiety linkage to the rhamnose C-2 hydroxyl position was clarified by the comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **1** as for **2**. The structure of **3** was determined to be (22*S*)-cholest-5-ene-3β,11β,16β,22-tetrol 16-*O*-(2-*O*-acetyl-α-L-rhamnopyranoside).

Compound **4** was obtained as a white amorphous powder, $[\alpha]_D$ −42° (methanol). The ^{13}C-NMR spectrum of **4** showed a pair of olefinic carbon signals at δ 132.4 (C) and 123.0 (CH) in addition to the C-5 (δ 142.9) and C-6 (δ 120.8) olefinic carbon signals. The ^{1}H-NMR spectrum of **4** showed two three-proton singlet signals at δ 1.69 and 1.67 (each 3H, s) attributable to methyl protons on the double bond, which were present as two three-proton doublet signals at δ 0.85 (3H × 2, d, J = 6.5 Hz) attributable to the H-26 and H-27 methyl protons in the ^{1}H-NMR spectrum of **1**. All other signals appeared at almost the same positions. Hydrogenation of **4** over 10% palladium on carbon furnished **1**. The above facts indicated that **4** was the corresponding 24-ene derivative of **1**, and the structure was elucidated as (22*S*)-cholesta-5,24-diene-

TABLE I. ^{13}C-NMR Spectral Data for **1**, **1a** and **2**—**5**[a]

C	1	1a	2	3	4	5
1	40.1	40.1	40.1	40.1	40.0	40.0
2	32.3	32.1[b]	32.3	32.3	32.3	32.3
3	71.7	71.7[c]	71.7	71.7	71.7	71.7
4	44.2	44.2	44.2	44.2	44.1	44.2
5	142.9	142.9	143.0	143.0	142.9	143.0
6	120.9	121.0	120.8	120.8	120.8	120.8
7	32.9	33.0	32.9	33.0	32.9	32.9
8	31.9	32.0	31.8	31.9	31.8	31.8
9	57.2	57.4	57.1	57.2	57.2	57.1
10	38.9	38.9	38.9	38.9	38.8	38.8
11	68.2	68.2	68.2	68.2	68.2	68.2
12	51.9	52.5	51.9	51.8	51.9	51.9
13	43.0	43.5	42.9	43.0	43.0	43.0
14	54.6	54.6	54.5	54.5	54.5	54.5
15	35.6	37.2	35.6	35.5	35.6	35.6
16	82.4	75.1	82.8	82.8	82.3	82.8
17	57.9	58.2	57.9	57.8	57.8	57.9
18	14.4	15.1	14.4	14.4	14.4	14.4
19	19.3	19.3	19.3	19.3	19.3	19.3
20	36.0	36.1	36.1	36.0	35.1	35.4
21	11.9	14.8	12.0	11.9	11.8	11.9
22	73.2	71.8[c]	72.6	72.5	73.1	72.2
23	34.4	32.5[b]	34.3	34.6	35.3	35.3
24	36.8	36.7	36.9	36.4	123.0	123.3
25	28.7	28.5	28.7	29.0	132.4	132.2
26	22.9	23.0	22.9	23.0	25.9	25.8
27	22.8	22.8	22.7	22.9	18.1	18.1
1′	104.9		104.8	101.5	104.9	104.9
2′	72.4		70.4	74.2	72.0	70.4
3′	72.7		76.3	70.8	72.6	76.2
4′	74.0		71.2	74.2	74.0	71.2
5′	71.0		71.0	71.0	70.9	71.0
6′	18.4		18.4	18.3	18.4	18.3
Ac			170.7	170.4		170.8
			21.1	21.0		21.1

a) Spectra were measured in pyridine-d_5. b, c) Assignments are interchangeable.

3β,11β,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside.

Compound **5** was a monoacetate of **4**, and the acetyl moiety linkage to the rhamnose C-3 hydroxyl position was confirmed by the ^{1}H- and ^{13}C-NMR spectra. The structure of **5** was determined to be (22*S*)-cholesta-5,24-diene-3β,11β,16β,22-tetrol 16-*O*-(3-*O*-acetyl-α-L-rhamnopyranoside).

Compounds **1**—**5** are new polyhydroxylated cholestane glycosides. A variety of polyhydroxylated sterols derived from a 24-methylcholestane skeleton have been reported in recent years from marine natural sources,[7] however, polyhydroxylated sterols from a cholestane skeleton are rare in nature.[2a,7a]

Compounds **1**, **1a** and **2**—**5** were evaluated for cyclic AMP phosphodiesterase inhibition.[8] Compounds **2** and **5** showed potent inhibitory activity (**2**: IC_{50} 9.9×10^{-5}; **5**: IC_{50} 10.9×10^{-5}), while **1**, **1a**, **3** and **4** were inactive. Acetyl group linked to the rhamnose C-3 hydroxyl position is believed to contribute to the inhibitory activity.

Experimental

Optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 instrument, and MS on a Hitachi M-80 or a VG AutoSpec E machine. NMR spectra were taken with a Bruker AM-400 spectrometer. Chemical shifts are given in δ-value referring to internal tetramethylsilane (TMS), and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad, dd=doublet of doublets, dq=doublet of quartets. Silica gel (Fuji Davison Co., Ltd.) and Sephadex LH-20 (Pharmacia Co., Ltd.) were used for column chromatographies. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 F_{254} S (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H_2SO_4 solution followed by heating. HPLC was performed with a Tosoh HPLC system (Tosoh Co., Ltd.: pump, CCPM; controller, CCP controller PX-8010; detector, RI-8010) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo Kasei Kogyo Co., Ltd., 10 mm i.d. × 250 mm, ODS, 5 μm).

Isolation The *n*-BuOH-soluble phase of the MeOH extract of the bulbs of *Ornithogalum saundersiae* (16.2 kg) was fractionated on a silica gel column with a gradient mixture of $CHCl_3$–MeOH to four fractions (I—IV) as described previously.[3] Fraction I was submitted to silica gel column chromatography with a gradient mixture of $CHCl_3$–MeOH (9:1, 6:1, 2:1) and HPLC with MeOH–H_2O (2:1) to yield compounds **1** (32.8 mg), **2** (56.9 mg), **3** (8.5 mg), **4** (32.0 mg) and **5** (28.0 mg).

(22*S*)-Cholest-5-ene-3β,11β,16β,22-tetrol 16-*O*-α-L-Rhamnopyranoside (1) A white amorphous powder, $[\alpha]_D^{28}$ −39° (*c*=0.50, MeOH). Negative-ion FAB-MS *m/z*: 579 $[M-H]^-$, 433 $[aglycon-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2945 (CH), 1470, 1455, 1385, 1265, 1125, 1050, 1020, 985, 965, 910, 805, 700. ^{1}H-NMR (pyridine-d_5) δ: 5.51 (1H, br d, *J*=4.9 Hz, H-6), 5.26 (1H, br s, H-1′), 4.47 (1H, br s, H-2′), 4.47—4.22 (5H, overlapping, H-11, H-16, H-3′, H-4′, H-5′), 3.99 (1H, br s, H-22), 3.92 (1H, m, H-3), 1.68 (3H, d, *J*=6.0 Hz, H-6′), 1.43 (3H, s, H-19), 1.23 (3H, d, *J*=6.8 Hz, H-21), 1.04 (3H, s, H-18), 0.85 (3H×2, d, *J*=6.5 Hz, H-26, H-27).

Acid Hydrolysis of 1 A solution of **1** (15.0 mg) in 1 N HCl (dioxane–H_2O, 1:1) was heated in a boiling water bath for 1 h. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (OH^- form) (Organo Co., Ltd.) column and purified by silica gel column chromatography with a gradient mixture of $CHCl_3$–MeOH (19:1, 9:1, 4:1, 2:1) to furnish L-rhamnose and an aglycon (**1a**) (9.0 mg). L-Rhamnose: $[\alpha]_D^{26}$ +11° (*c*=0.04, H_2O). TLC *Rf* 0.65 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). Compound **1a**: A white amorphous powder, $[\alpha]_D^{26}$ −44° (*c*=0.30, MeOH). EI-MS *m/z* (%): 434 $[M]^+$ (2.5), 416 $[M-H_2O]^+$ (2.5), 401 (3), 398 $[M-2H_2O]^+$ (2.5), 383 (3), 380 $[M-3H_2O]^+$ (2), 371 (3.5), 363 (2.5), 355 (2.5), 345 (3), 334 (5), 316 (45), 298 (100), 287 (22), 283 (20), 280 (12), 269 (16), 265 (21), 211 (17), 197 (19), 171 (25), 160 (39), 145 (77), 131 (47), 105 (71). IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 2930 and 2855 (CH), 1460, 1375, 1355, 1255, 1020, 950, 800. ^{1}H-NMR (pyridine-d_5) δ: 5.50 (1H, br d, *J*=5.3 Hz, H-6), 4.77 (1H, ddd, *J*=7.6, 7.6, 4.6 Hz, H-16), 4.35 (1H, br, H-11), 4.13 (1H, br dd, *J*=8.2, 1.9 Hz, H-22), 3.88 (1H, m, H-3), 2.56 (1H, ddq, *J*=11.2, 1.9, 7.0 Hz, H-20), 1.73 (1H, dd, *J*=11.2, 7.6 Hz, H-17), 1.41 (3H, s, H-19), 1.25 (3H, s, H-18), 1.21 (3H, d, *J*=7.0 Hz, H-21), 0.89 (3H, d, *J*=6.6 Hz, H-26 or H-27), 0.88 (3H, d, *J*=6.6 Hz, H-26 or H-27).

Preparation of (*R*)-MTPA Ester (1b) of 1a Compound **1a** (2.8 mg) was dissolved in dry CH_2Cl_2 (0.8 ml) and pyridine (0.2 ml), to which was added a large amount of (*R*)-MTPA-Cl and 4-(dimethylamino)pyridine (10 mg), and the solution was allowed to stand at room temperature for 1 h. The reaction mixture, after dilution with H_2O, was extracted with Et_2O and subjected to silica gel column chromatography with hexane–Me_2CO (4:1) to yield (*R*)-MTPA ester (**1b**) (4.0 mg) of **1a**. Compound **1b**: A white amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3445 (OH), 2955, 2930 and 2850 (CH), 1740 (C=O), 1465, 1450, 1380, 1260, 1165, 1120, 1075, 1020, 960, 800, 715, 695. ^{1}H-NMR (chloroform-d_1) δ: 7.64—7.29 (10H, aromatic protons), 5.47 (1H, br d, *J*=4.8 Hz, H-6), 5.39 (1H, br dd, *J*=7.0, 7.0 Hz, H-22), 4.88 (1H, m, H-3), 4.36 (1H, br s, H-16), 3.98 (1H, br, H-11), 3.57 (3H×2, s, OMe×2), 2.17 (1H, H-17), 2.14 (1H, H-20), 1.62 (1H, H-23a), 1.53 (3H, s, H-19), 1.52 (1H, H-23b), 1.15 (3H, s, H-18), 0.93 (3H, d, *J*=6.9 Hz, H-21), 0.89 (3H, d, *J*=6.6 Hz, H-26 or H-27), 0.87 (3H, d, *J*=6.6 Hz, H-26 or H-27).

Preparation of (*S*)-MTPA Ester (1c) of 1a The (*S*)-MTPA ester (**1c**) (3.3 mg) was prepared from **1a** (2.7 mg) and (*S*)-MTPA-Cl according to the method described for the preparation of the (*R*)-MTPA ester (**1b**) of **1a**. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2960 and 2870 (CH), 1740 (C=O), 1465, 1450, 1385, 1265, 1165, 1120, 1080, 1020, 960, 855, 805, 765, 710, 700. ^{1}H-NMR (chloroform-d_1) δ: 7.62—7.35 (10H, aromatic protons), 5.48 (1H, br d, *J*=5.3 Hz, H-6), 5.41 (1H, br dd, *J*=7.0, 7.0 Hz, H-22), 4.89 (1H, m, H-3), 4.42 (1H, br s, H-16), 4.01 (1H, br, H-11), 3.57 (3H, s, OMe), 3.51 (3H, s, OMe), 2.20 (1H, H-17), 2.17 (1H, H-20), 1.54 (1H, s, H-19), 1.53 (3H, s, H-23a), 1.45 (1H, H-23b), 1.18 (3H, s, H-18), 0.97 (3H, d, *J*=6.9 Hz, H-21), 0.86 (3H, d, *J*=6.6 Hz, H-26 or H-27), 0.84 (3H, d, *J*=6.6 Hz, H-26 or H-27).

(22*S*)-Cholest-5-ene-3β,11β,16β,22-tetrol 16-*O*-(3-*O*-Acetyl-α-L-rhamnopyranoside) (2) A white amorphous powder, $[\alpha]_D^{28}$ −39° (*c*=0.50, MeOH). Negative-ion FAB-MS *m/z*: 621 $[M-H]^-$, 579 $[M-Ac]^-$, 433 $[aglycon-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1720 (C=O), 1465, 1375, 1245, 1120, 1045, 980, 960, 805, 695. ^{1}H-NMR (pyridine-d_5) δ: 5.75 (1H, dd, *J*=9.6, 2.7 Hz, H-3′), 5.51 (1H, br d, *J*=4.9 Hz, H-6), 5.24 (1H, br s, H-1′), 4.59 (1H, br s, H-2′), 4.53—4.23 (4H, overlapping, H-11, H-16, H-4′, H-5′), 4.01 (1H, br s, H-22), 3.92 (1H, m, H-3), 1.94 (3H, s, Ac), 1.71 (3H, d, *J*=6.0 Hz, H-6′), 1.42 (3H, s, H-19), 1.22 (3H, d, *J*=6.9 Hz, H-21), 1.02 (3H, s, H-18), 0.91 (3H, d, *J*=6.0 Hz, H-26 or H-27), 0.90 (3H, d, *J*=6.2 Hz, H-26 or H-27).

Alkaline Hydrolysis of 2 Compound **2** (27.0 mg) was treated with 3% NaOMe in MeOH at room temperature for 1 h. The reaction mixture was neutralized by passing it through an Amberlite IR-120B column (Organo Co., Ltd.), and the eluate was subjected to Sephadex LH-20 column chromatography with MeOH to yield **1** (20.5 mg), which was identified by the ^{1}H-NMR spectrum and direct TLC comparison. TLC (RP-18): *Rf* 0.41 (MeOH–H_2O, 2:1).

(22*S*)-Cholest-5-ene-3β,11β,16β,22-tetrol 16-*O*-(2-*O*-Acetyl-α-L-rhamnopyranoside) (3) A white amorphous powder, $[\alpha]_D^{28}$ −37° (*c*=0.50, MeOH). Negative-ion FAB-MS *m/z*: 621 $[M-H]^-$, 579 $[M-Ac]^-$, 433 $[aglycon-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2935 (CH), 1725 (C=O), 1465, 1370, 1240, 1110, 1050, 1020, 980, 965, 905, 805, 695. ^{1}H-NMR (pyridine-d_5) δ: 5.64 (1H, br d, *J*=2.8 Hz, H-2′), 5.51 (1H, br d, *J*=5.1 Hz, H-6), 5.05 (1H, br s, H-1′), 4.54 (1H, dd, *J*=9.2, 2.8 Hz, H-3′), 4.41 (1H, ddd, *J*=7.4, 7.4, 4.1 Hz, H-16), 4.32 (1H, br, H-11), 4.28 (1H, dq, *J*=9.2, 6.1 Hz, H-5′), 4.18 (1H, dd, *J*=9.2, 9.2 Hz, H-4′), 4.05 (1H, br s, H-22), 3.92 (1H, m, H-3), 2.02 (3H, s, Ac), 1.70 (3H, d, *J*=6.1 Hz, H-6′), 1.44 (3H, s, H-19), 1.24 (3H, d, *J*=6.9 Hz, H-21), 1.04 (3H, d, *J*=6.3 Hz, H-26 or H-27), 1.01 (3H, s, H-18), 1.00 (3H, d, *J*=6.5 Hz, H-26 or H-27).

Alkaline Hydrolysis of 3 Compound **3** (1.0 mg) was hydrolyzed with 3% NaOMe in MeOH as for **2** to yield **1**, which was identified by direct TLC comparison.

(22*S*)-Cholesta-5,24-diene-3β,11β,16β,22-tetrol 16-*O*-α-L-Rhamnopyranoside (4) A white amorphous powder, $[\alpha]_D^{28}$ −42° (*c*=0.54, MeOH). Negative-ion FAB-MS *m/z*: 577 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2975 and 2940 (CH), 1445, 1380, 1260, 1120, 1045, 1020, 980, 960, 910, 825, 810, 695. ^{1}H-NMR (pyridine-d_5) δ: 5.53 (1H, br t, *J*=7.0 Hz, H-24), 5.50 (1H, br d, *J*=5.0 Hz, H-6), 5.23 (1H, br s, H-1′), 4.48 (1H, br s, H-2′), 4.47—4.22 (5H, overlapping, H-11, H-16, H-3′, H-4′, H-5′), 4.10 (1H, br s, H-22), 3.91 (1H, m, H-3), 1.69 (3H, s, H-27), 1.68 (3H, d, *J*=6.0 Hz, H-6′), 1.67 (3H, s, H-26), 1.43 (3H, s, H-19), 1.26 (3H, d, *J*=6.8 Hz, H-21), 1.03 (3H, s, H-18).

Catalytic Hydrogenation of 4 A mixture of **4** (15.0 mg) and 10% palladium on carbon (10 mg) in methanol was stirred under an H_2 atmosphere at ambient temperature for 30 min. The reaction mixture, after removal of palladium on carbon by filtration, was subjected to HPLC with MeOH–H_2O (2:1) to yield **1** (4.8 mg), which was identified by the ^{1}H-NMR spectrum and direct TLC comparison.

(22*S*)-Cholesta-5,24-diene-3β,11β,16β,22-tetrol 16-*O*-(3-*O*-Acetyl-α-L-rhamnopyranoside) (5) A white amorphous powder, $[\alpha]_D^{28}$ −33° (*c*=0.50, MeOH). Negative-ion FAB-MS *m/z*: 619 $[M-H]^-$, 577 $[M-Ac]^-$, 431 $[aglycon-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2975 and 2935 (CH), 1725 (C=O), 1445, 1380, 1255, 1110, 1050, 1025, 985, 965, 885, 810, 700. ^{1}H-NMR (pyridine-d_5) δ: 5.76 (1H, dd, *J*=9.7, 2.9 Hz, H-3′), 5.57 (1H, br t, *J*=7.0 Hz, H-24), 5.51 (1H, br d, *J*=5.0 Hz, H-6), 5.21 (1H, br s, H-1′), 4.60 (1H, br s, H-2′), 4.52—4.24 (4H, overlapping, H-11, H-16, H-4′, H-5′), 4.10 (1H, br s, H-22), 3.91 (1H, m, H-3), 1.94 (3H, s, Ac), 1.71 (3H, s, H-27), 1.70 (3H, d, *J*=6.1 Hz, H-6′), 1.67 (3H, s, H-26), 1.42 (3H, s, H-19), 1.24 (3H, d, *J*=6.9 Hz, H-21), 1.00 (3H, s, H-18).

Alkaline Hydrolysis of 5 Compound **5** (5.0 mg) was hydrolyzed with 3% NaOMe in MeOH as for **2** to yield **4** (4.1 mg), which was identified by the ^{1}H-NMR spectrum and direct TLC comparison. TLC (RP-18): *Rf* 0.50 (MeOH–H_2O, 2:1).

Assay of Cyclic AMP Phosphodiesterase Activity The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described in a previous paper.[8a]

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