

New Cholestane Bidesmosides from the Bulbs of *Ornithogalum thyrsoides*

Satoshi KUBO, Yoshihiro MIMAKI, Yutaka SASHIDA,*

Tamotsu NIKAIIDO,[†] and Taichi OHMOTO[†]

Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03

[†]Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274

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Four new cholestane bidesmosides have been isolated from the fresh bulbs of *Ornithogalum thyrsoides*. Their structures were determined by spectroscopic analysis and chemical evidence to be (22*S*)-5 α -cholestane-1 β ,3 β ,16 β ,22-tetrol 1-*O*- α -L-rhamnopyranoside 16-*O*- β -D-glucopyranoside (**1**), (22*S*)-5 α -cholestane-1 β ,3 β ,16 β ,22-tetrol 1-*O*- α -L-rhamnopyranoside 16-*O*-(6-*O*-acetyl- β -D-glucopyranoside) (**2**), (22*S*)-5 α -cholest-24-ene-1 β ,3 β ,16 β ,22-tetrol 1-*O*- α -L-rhamnopyranoside 16-*O*- β -D-glucopyranoside (**3**), and (22*S*)-5 α -cholest-24-ene-1 β ,3 β ,16 β ,22-tetrol 1-*O*- α -L-rhamnopyranoside 16-*O*-(6-*O*-acetyl- β -D-glucopyranoside) (**4**), respectively. An advanced Mosher's method was applied to determine the C-22 absolute configuration. Compound **4** showed inhibitory activity on cyclic AMP phosphodiesterase.

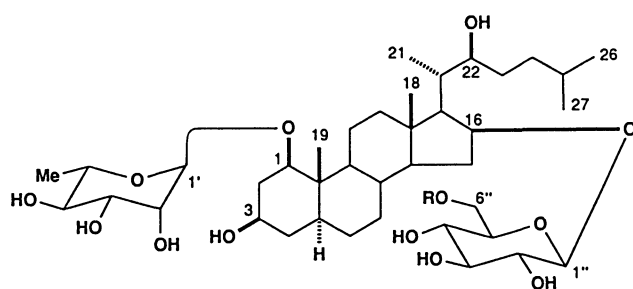
The genus *Ornithogalum* belonging to Liliaceae contains some 150 species, and has a distribution in the temperate climates of Europe, Asia, and Africa.¹⁾ Some *Ornithogalum* plants are known to be poisonous plants, and several cardenolide glycosides were isolated and identified.²⁾

O. thyrsoides is native to southwestern Cape Province. The poisoning of stock sometimes occurs when the plant is accidentally included in cut forage.¹⁾ No chemical analysis, however, has been carried out on the

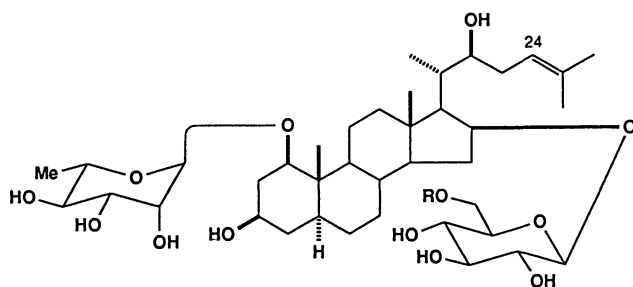
plant. As part of a systematic study on the constituents of the bulbs of Liliaceae plants,³⁾ we undertook an investigation of the methanolic extract of *O. thyrsoides*, resulting in the isolation of four new cholestanol glycosides. The structures of the new compounds were determined by spectroscopic analysis and chemical evidence. The inhibitory activity on cyclic AMP phosphodiesterase of the isolated compounds was assayed.

A methanolic extract of the fresh bulbs of *O. thyrsoides* was partitioned between H₂O and chloroform, and then between H₂O and 1-butanol. Compounds **1**–**4** were isolated from the 1-butanol-soluble phase after repeated chromatographic separations.

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D -29.0^\circ$ (methanol). The secondary ion (SI) mass spectrum showed an $[M+Na]^+$ ion at m/z 767, corresponding to the molecular formula, C₃₉H₆₈O₁₃. The IR spectrum of **1** showed an absorption band of hydroxyl groups at 3382 cm⁻¹. Acid hydrolysis of **1** with 1 M (1 M=1 mol dm⁻³) hydrochloric acid (dioxane-H₂O) yielded D-glucose and L-rhamnose as the carbohydrate compounds, and an aglycon (**1a**), C₂₇H₄₈O₄. The ¹H NMR spectrum of **1a** showed two three-proton singlet signals at $\delta=1.22$ and 1.15, indicating the presence of two angular methyl groups, as well as three three-proton doublet signals at $\delta=1.18$ ($J=7.0$ Hz) and 0.89 (3H $\times$ 2, $J=6.5$ Hz) due to CH₃-CH groups. The ¹³C NMR spectrum of **1a** displayed a total of 27 signals, which were assigned as CH₃ $\times$ 5, CH₂ $\times$ 9, CH $\times$ 11, and C $\times$ 2 with the aid of the DEPT spectra. The CH signals at $\delta=77.3$, 75.4, 71.5, and 68.0 were apparently due to carbons bearing oxygen functions. The above-mentioned data indicated that **1a** was a cholestanetetrol. The location of the four hydroxyl groups were determined by the following spectral data. A multiplet proton signal centered at $\delta=3.96$ ($W_{1/2}=23.5$ Hz) was unequivocally assigned to the H-3 α axial proton geminately bearing hydroxyl group. A hydroxymethine proton coupled with the adjacent methylene protons



R
1 H
2 Ac



R
3 H
4 Ac

Table 1. ^{13}C NMR Spectral Data for Compounds **1**, **1a**, 5α -Cholestan- 3β -ol, **2**, **3**, and **4**

C	1	1a	5α -cholestan- 3β -ol	2	3	4
1	81.9	75.4	37.6	81.9	81.9	82.0
2	37.4	44.1	32.5 ^{a)}	37.4	37.4	37.4
3	67.6	68.0	70.7	67.7	67.6	67.7
4	39.5	39.7	39.3	39.6	39.5	39.5
5	42.9	43.1	45.3	43.0	42.9	43.0
6	29.0	29.2	29.2	29.0	29.0	29.0
7	32.0	32.5	32.4 ^{a)}	32.1	32.0	32.1
8	36.4	36.4	35.8	36.6	36.4	36.6
9	55.1	55.1	54.7	55.2	55.1	55.2
10	41.6	42.7	35.8	41.7	41.6	41.7
11	24.5	24.8	21.6	24.5	24.5	24.5
12	40.9	41.5	40.4	41.0	40.9	41.0
13	42.3	42.0	42.9	42.4	42.4	42.4
14	55.2	55.9	56.7 ^{b)}	55.2	55.2	55.2
15	37.3	37.5	24.5	37.4	37.1	37.2
16	82.6	77.3	28.6	82.9	82.5	82.7
17	58.2	58.5	56.6 ^{b)}	58.2	58.2	58.2
18	14.0	15.3	12.3	14.0	14.1	14.0
19	8.1	7.6	12.6	8.1	8.1	8.1
20	36.0	36.2	36.1	36.0	35.5	35.5
21	12.6	13.9	19.0	12.5	12.6	12.5
22	73.2	71.5	36.5	73.2	72.8	72.9
23	33.7	32.0	24.2	33.7	34.7	34.8
24	36.8	36.8	39.8	36.8	124.6	124.6
25	28.9	28.5	28.3	28.9	131.4	131.4
26	23.0 ^{a)}	22.8 ^{a)}	22.7 ^{c)}	23.0 ^{a)}	26.0	26.0
27	23.1 ^{a)}	23.0 ^{a)}	22.9 ^{c)}	23.1 ^{a)}	18.3	18.3
1'	98.5			98.5	98.5	98.6
2'	72.8			72.9	72.8	72.8
3'	73.2			73.2	73.2	73.3
4'	73.8			73.8	73.8	73.8
5'	71.0			71.0	71.0	71.0
6'	18.7			18.7	18.7	18.7
1''	106.9			106.9	106.8	106.7
2''	75.7			75.4	75.7	75.5
3''	78.7			78.5	78.7	78.5
4''	71.8			71.9	71.8	71.9
5''	78.1			75.0	78.2	75.0
6''	62.9			64.9	62.9	64.9
Ac				170.7		170.7
				20.9		20.9

Spectra were measured in pyridine- d_5 . a—c) Assignments may be reversed.

appeared at $\delta=3.71$ as a doublet of doublets signal ($J=11.2$ and 4.3 Hz). Further, the ^{13}C signals arising from the C-2, C-10, and C-11 were displaced downfield, while the signal from the C-19 moved upfield as compared with those of 5α -cholestan- 3β -ol, indicating the presence of the 1β -hydroxyl group.⁴⁾ The presence of the 1β - and 3β -hydroxyl groups, and the ring junction being A/B *trans*, were supported by an agreement of the ^{13}C NMR signals due to the A and B rings between **1a** and (25*S*)- 5α -spirostan- $1\beta,3\beta$ -diol.⁴⁾ The H-18 and H-21 methyl protons resonated at $\delta=1.15$ and 1.18 , which were moved to lower field by 0.47 and 0.19 ppm, respectively, as compared with those of 5α -cholestan- 3β -ol.⁵⁾ Also, the ^{13}C signals due to the D-ring and the side-chain agreed with those of (22*S*)-cholest-5-ene-

$3\beta,16\beta,22$ -triol.⁶⁾ The remaining two hydroxyl groups were shown to be located at the C-16 β and C-22 positions. The absolute configuration of the C-22 position was determined by an advanced Mosher's method.⁷⁾ Compound **1a** was treated with (*R*)- and (*S*)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) to yield $3\beta,22$ -bis-MTPA esters. A comparison of the ^1H NMR spectrum of the (*R*)-MTPA ester (**1b**) with that of the (*S*)-MTPA ester (**1c**) revealed that the H-23 methylene proton, and H-26 and H-27 methyl proton signals of **1b**, appeared at lower field than those of **1c**, and that the H-20 methine proton, as well as H-21, H-18, and H-19 methyl proton signals of **1b** appeared at higher fields than those of **1c**, as shown in Fig. 1. The MTPA moiety of the C-3 β hydroxyl group has no influence on the H-18 and H-19 methyl, and side-chain protons.⁷⁾ Thus, the structure of **1a**, including the absolute configuration of the C-22 position, was confirmed to be (22*S*)- 5α -cholestan- $1\beta,3\beta,16\beta,22$ -tetrol. The ^{13}C NMR spectrum of **1** showed the presence of a terminal β -D-glucopyranosyl unit and a terminal α -L-rhamnopyranosyl unit in the molecule. The linkage position between each monosaccharide and the aglycon was established by observations of the ^1H - ^{13}C long-range coupling from each anomeric proton across the glycosidic bond to the carbon of the aglycon. In the ^1H - ^{13}C COSY spectrum of **1**, the carbon signals at $\delta=82.6$ and 81.9 showed one-bond ^1H - ^{13}C correlations with the proton signals at $\delta=4.50$ (ddd, $J=7.0, 7.0$, and

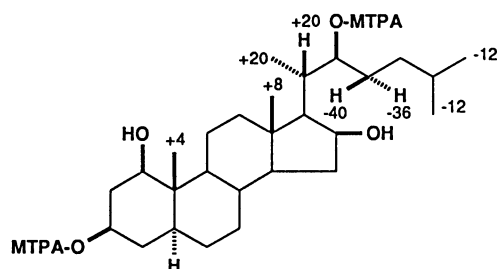


Fig. 1. Chemical shift differences between **1a** (*S*)-MTPA ester and **1a** (*R*)-MTPA ester.

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

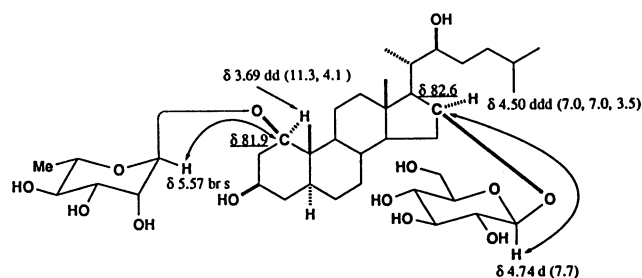


Fig. 2. ^1H - ^{13}C Long-range correlations of **1** in pyridine- d_5 . J values (Hz) in the ^1H NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C NMR chemical shifts.

3.5 Hz, H-16 α) and 3.69 (dd, $J=11.3$ and 4.1 Hz, H-1 α). The anomeric proton signals of the glucose at $\delta=4.74$ (d, $J=7.7$ Hz) and the rhamnose at $\delta=5.57$ (br s) showed long-range correlations with the carbon signals at $\delta=82.6$ (C-16) and 81.9 (C-1), respectively, in the ^1H -Detected Multiple-Bond Heteronuclear Multiple Bond Connectivity (HMBC) spectrum. Accordingly, the structure of **1** was elucidated to be (22*S*)-5 α -cholestane-1 β ,3 β ,16 β ,22-tetrol 1-*O*- α -L-rhamnopyranoside 16-*O*- β -D-glucopyranoside.

The spectral properties of **2** were essentially identical with those of **1**. The fast-atom-bombardment (FAB) mass spectrum showed an $[\text{M}+\text{Na}]^+$ ion at m/z 809 which exceeded that of **1** by 42 mass units. The IR, ^1H NMR, and ^{13}C NMR spectra of **2** indicated the presence of an acetyl group in the molecule (IR: 1727 cm^{-1} ; ^1H NMR: $\delta=2.08$, 3H, s; ^{13}C NMR: $\delta=170.7$ and 20.9). Treatment of **2** with 3% sodium methoxide in methanol yielded **1**. Therefore, **2** must be a monoacetate of **1**. In the ^{13}C NMR spectrum of **2**, the signal due to the glucose C-6 position was shifted to lower field by 2.0 ppm, whereas the signal due to the C-5 to upper field by 3.1 ppm as compared with those of **1**, clearly accounting for the acetyl moiety linkage to the glucose C-6 hydroxyl position. Thus, the structure of **2** was elucidated as being (22*S*)-5 α -cholestane-1 β ,3 β ,16 β ,22-tetrol 1-*O*- α -L-rhamnopyranoside 16-*O*-(6-*O*-acetyl- β -D-glucopyranoside).

Compound **3** was also a cholestane bisdesmoside. The ^1H NMR spectrum of **3** showed two three-proton singlet signals at $\delta=1.74$ and 1.68 (each 3H, s) assignable to methyl groups on a double bond, which were present as three-proton doublet signals at $\delta=0.93$ (3H, d, $J=6.1$ Hz) and 0.92 (3H, d, $J=5.9$ Hz) assignable to the H-26 and H-27 methyl groups in **1**. All other signals remained almost unaffected. Hydrogenation of **3** over 10% palladium on carbon yielded **1**. The above-mentioned data indicated that **3** was the corresponding 24-ene derivative of **1**; the structure was elucidated as being (22*S*)-5 α -cholest-24-ene-1 β ,3 β ,16 β ,22-tetrol 1-*O*- α -L-rhamnopyranoside 16-*O*- β -D-glucopyranoside.

Compound **4** was a monoacetate of **3**, which was confirmed by the IR, ^1H NMR, and ^{13}C NMR spectra, as well as by alkaline hydrolysis. In the ^{13}C NMR spectrum of **4**, it was observed that the signal of the glucose C-6 position was displaced downfield by 2.0 ppm, and that the signal of the C-5 upfield by 3.2 ppm, compared with those of **3**, indicating that the C-6 hydroxyl position of the glucose is the acylated position to which acetic acid is linked. The structure of **4** was elucidated as being (22*S*)-5 α -cholest-24-ene-1 β ,3 β ,16 β ,22-tetrol 1-*O*- α -L-rhamnopyranoside 16-*O*-(6-*O*-acetyl- β -D-glucopyranoside).

Compounds **1**–**4** are new cholestane bisdesmosides. (22*S*)-Hydroxycholestane derivatives⁶⁾ and cholestane bisdesmosides^{6,8)} are very rare in nature.

Compounds **1**–**4** were evaluated for cyclic AMP phosphodiesterase inhibition.⁹⁾ Compound **4** showed

inhibitory activity (IC_{50} 15.3×10^{-5} M), while **1**–**3** were inactive ($\text{IC}_{50} > 500 \times 10^{-5}$ M). Cholesterol glycosides isolated from other Liliaceae plants are now being assayed.

Experimental

Optical rotations were measured with a JASCO DIP-360 automatic polarimeter. IR spectra were recorded on a Perkin-Elmer 1710 FT-IR instrument, and MS on a Hitachi M-80 or a VG AutoSpec E machine. 1D NMR spectra (ppm, J Hz) were taken with a Bruker AM-400, and 2D NMR spectra with a Bruker AM-500 spectrometer employing the standard Bruker software. HPLC was performed on 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. $\times$ 250 mm, ODS, 5 μm).

Extraction and Isolation. Fresh bulbs of *O. thyrsoides* (4.5 kg) were cut into pieces and extracted with hot MeOH. The MeOH extract was concentrated under reduced pressure. The dark viscous concentrate was partitioned between H_2O and CHCl_3 , and then between H_2O and *n*-BuOH. Column chromatography of the *n*-BuOH extract on silica gel with CHCl_3 -MeOH, with increasing proportion of MeOH, gave four fractions. Fraction 3 was further fractionated by passing it through a Diaion HP-20 column with H_2O gradually enriched with MeOH. The 50% MeOH- H_2O , 80% MeOH- H_2O , and MeOH eluate fractions were combined and chromatographed on silica gel with a gradient mixture of CHCl_3 -MeOH, Sephadex LH-20 with MeOH, and on ODS with MeOH- H_2O (2:1) to give compounds **2** and **4** as almost pure compounds. Final purification of **2** and **4** was carried out by preparative HPLC with MeOH- H_2O (3:1); **2** (102 mg) and **4** (19.5 mg).

Compounds **1** (177 mg) and **3** (60.0 mg) were isolated from fraction 4 after similar chromatographic separations as for fraction 3.

Compound 1. A white amorphous powder, $[\alpha]_{\text{D}}^{26} -29.0^\circ$ (c 0.17, MeOH). SIMS m/z 767 $[\text{M}+\text{Na}]^+$; IR (Film) 3382 (OH), 2933 and 2880 (CH), 1465, 1447, 1420, 1384, 1370, 1265, 1230, 1165, 1075, 1039, 990, 960, 910, 840, and 805 cm^{-1} ; ^1H NMR (pyridine- d_5) $\delta=5.57$ (1H, br s, H-1'), 4.74 (1H, d, $J=7.7$ Hz, H-1'), 4.50 (1H, ddd, $J=7.0$, 7.0 and 3.5 Hz, H-16), 4.47 (1H, br s, H-2'), 4.46 (1H, br d, $J=11.5$ Hz, H-6'a), 4.37 (1H, dd, $J=11.5$ and 4.7 Hz, H-6'b), 4.32–4.07 (5H, H-22, H-3', H-4', H-3'' and H-4''), 4.00 (1H, dd, $J=8.8$ and 7.7 Hz, H-2''), 3.90–3.79 (2H, H-3 and H-5''), 3.69 (1H, dd, $J=11.3$ and 4.1 Hz, H-1), 1.67 (3H, d, $J=5.8$ Hz, H-6'), 1.15 (3H, d, $J=7.0$ Hz, H-21), 0.99 (3H, s, H-19), 0.96 (3H, s, H-18), 0.93 (3H, d, $J=6.1$ Hz, H-26 or H-27), and 0.92 (3H, d, $J=5.9$ Hz, H-26 or H-27).

Acid Hydrolysis of 1. Hydrolysis of **1** (100 mg) with 1 M HCl (dioxane- H_2O , 1:1) was carried out at 100°C for 1 h. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (an anion-exchange resin) column, and purified by silica-gel column chromatography with CHCl_3 -MeOH (19:1) and CHCl_3 -MeOH- H_2O (20:10:1) to yield L-rhamnose (16.0 mg), D-glucose (11.9 mg), and an aglycon (**1a**) (25.0 mg). L-Rhamnose: $[\alpha]_{\text{D}}^{26} +10.1^\circ$ (c 0.12, H_2O); TLC, R_f 0.67 (*n*-BuOH- Me_2CO - H_2O , 4:5:1). D-Glucose: $[\alpha]_{\text{D}}^{26} +50.0^\circ$ (c 0.24, H_2O); TLC, R_f 0.33 (*n*-BuOH- Me_2CO - H_2O , 4:5:1). Compound **1a**: A white amorphous powder, $[\alpha]_{\text{D}}^{26}$

–15.9° (c 0.34, pyridine). CIMS m/z (%) 419 $[M-OH]^+$ (17), 401 (100), 383 (65), 365 (14), 318 (69), 303 (26), 289 (63), 273 (40), and 250 (13); IR (Film) 3333 (OH), 2936 and 2870 (CH), 1466, 1450, 1384, 1370, 1350, 1280, 1261, 1216, 1169, 1115, 1075, 1034, 1010, 957, and 908; 1H MMR (pyridine- d_5) δ =4.76 (1H, ddd, J =6.9, 6.9 and 4.4 Hz, H-16), 4.14 (1H, br d, J =7.6 Hz, H-22), 3.96 (1H, br m, $W_{1/2}$ =23.5 Hz, H-3), 3.71 (1H, dd, J =11.2 and 4.3 Hz, H-1), 1.22 (3H, s, H-19), 1.18 (3H, d, J =7.0 Hz, H-21), 1.15 (3H, s, H-18), and 0.89 (3H $\times$ 2, d, J =6.5 Hz, H-26 and H-27).

Preparation of (R)-MTPA Ester (1b) of 1a. Compound **1a** (5 mg) was dissolved in dry CH_2Cl_2 (0.8 ml) and pyridine (0.2 ml), to which was added large excess 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**) (6 mg) of **1a**. Compound **1b**: A white amorphous powder. IR (Film) 3585 (OH), 2951, 2870 and 2850 (CH), 1741 (C=O), 1470, 1451, 1390, 1271, 1168, 1123, 1110, 1081, 1021, 994, 766, 719, and 697 cm^{-1} ; 1H NMR ($CDCl_3$) δ =7.62–7.35 (10H, aromatic protons), 5.41 (1H, br dd, J =7.0 and 7.0 Hz, H-22), 4.99 (1H, m, H-3), 4.31 (1H, ddd, J =8.0, 8.0 and 3.7 Hz, H-16), 3.57 and 3.54 (each 3H, s, OMe), 3.56 (1H, overlapping with methoxyl protons, H-1), 2.14 (1H, H-20), 1.71 (1H, H-23a), 1.59 (1H, H-23b), 0.88 (3H, d, J =6.9 Hz, H-21), 0.87 (3H, d, J =7.4 Hz, H-26 or H-27), 0.85 (3H, d, J =6.6 Hz, H-26 or H-27), 0.84 (3H, s, H-19), and 0.81 (3H, s, H-18).

Preparation of (S)-MTPA Ester (1c) of 1a. The (S)-MTPA ester (**1c**) (3 mg) was prepared from **1a** (2.5 mg) and (S)-MTPA-Cl according to the method described for the preparation of the (R)-MTPA ester (**1b**) of **1**. Compound **1c**: A white amorphous powder. IR (Film) 3584 (OH), 2953, 2930 and 2850 (CH), 1741 (C=O), 1470, 1452, 1390, 1262, 1168, 1123, 1110, 1082, 1020, 995, 800, 765, and 698 cm^{-1} ; 1H NMR ($CDCl_3$) δ =7.64–7.36 (10H, aromatic protons), 5.42 (1H, br dd, J =6.9 and 6.9 Hz, H-22), 4.98 (1H, m, H-3), 4.38 (1H, ddd, J =7.1, 7.1 and 4.6 Hz, H-16), 3.56 and 3.50 (each 3H, s, OMe), 3.53 (1H, overlapping with methoxyl protons, H-1), 2.19 (1H, H-20), 1.61 (1H, H-23a), 1.50 (1H, H-23b), 0.93 (3H, d, J =6.9 Hz, H-21), 0.85 (3H, s, H-19), 0.84 (3H, d, J =7.5 Hz, H-26 or H-27), 0.83 (3H, s, H-18), and 0.82 (3H, d, J =6.6 Hz, H-26 or H-27).

Compound 2. A white amorphous powder, $[\alpha]_D^{26}$ –17.9° (c 0.15, MeOH). FABMS m/z 809 $[M+Na]^+$; IR (Film) 3386 (OH), 2936 and 2880 (CH), 1727 (C=O), 1465, 1450, 1415, 1385, 1368, 1340, 1251, 1175, 1130, 1080, 1038, 915, 840, and 805 cm^{-1} ; 1H NMR (pyridine- d_5) δ =5.58 (1H, br s, H-1'), 4.65 (1H, d, J =7.7 Hz, H-1''), 3.72 (1H, dd, J =11.4 and 4.0 Hz, H-1), 2.08 (3H, s, Ac), 1.68 (3H, d, J =5.7 Hz, H-6'), 1.13 (3H, d, J =7.0 Hz, H-21), 0.98 (3H $\times$ 2, s, H-18 and H-19), 0.94 (3H, d, J =6.0 Hz, H-26 or H-27), and 0.93 (3H, d, J =6.1 Hz, H-26 or H-27).

Alkaline Hydrolysis of 2. Compound **2** (20 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 (a cation exchange resin) column; the eluate was subjected to preparative HPLC with MeOH– H_2O (3:1) to yield **1** (5.2 mg), which was identified by the IR and 1H NMR spectra, as well as by a direct TLC comparison. Reversed-phase (ODS) TLC: R_f 0.66 (MeOH– H_2O , 3:1).

Compound 3. A white amorphous powder, $[\alpha]_D^{26}$ –30.8° (c 0.17, MeOH). SIMS m/z 765 $[M+Na]^+$ and 743 $[M+H]^+$; IR (Film) 3356 (OH), 2926, 2885 and 2860 (CH), 1445, 1415, 1382, 1375, 1360, 1265, 1165, 1120, 1075, 1037, 995, 915, 845, and 805 cm^{-1} ; 1H NMR (pyridine- d_5) δ =5.86 (1H, t, J =6.8 Hz, H-24), 5.57 (1H, br s, H-1'), 4.74 (1H, d, J =7.7 Hz, H-1''), 3.69 (1H, dd, J =11.3 and 4.2 Hz, H-1), 1.74 (3H, s, H-27), 1.68 (3H, s, H-26), 1.67 (3H, d, J =5.2 Hz, H-6'), 1.18 (3H, d, J =7.0 Hz, H-21), 0.98 (3H, s, H-18 or H-19), and 0.95 (3H, s, H-18 or H-19).

Catalytic Hydrogenation of 3. A mixture of **3** (10 mg) and 10% palladium on carbon (10 mg) in methanol was stirred under an H_2 atmosphere at ambient temperature overnight. The reaction mixture, after removal of palladium on carbon by filtration, was subjected to preparative HPLC with MeOH– H_2O –2-methoxyethanol (20:10:1) to yield **1** (5 mg), which was identified by the IR and 1H NMR spectra, as well as by a direct TLC comparison.

Compound 4. A white amorphous powder, $[\alpha]_D^{26}$ –20.5° (c 0.15, MeOH). FABMS m/z 807 $[M+Na]^+$; IR (Film) 3355 (OH), 2922 and 2960 (CH), 1725 (C=O), 1450, 1420, 1370, 1340, 1260, 1235, 1175, 1080, 1037, 915, 845, and 805 cm^{-1} ; 1H NMR (pyridine- d_5) δ =5.86 (1H, t, J =6.6 Hz, H-24), 5.58 (1H, br s, H-1'), 4.66 (1H, d, J =7.7 Hz, H-1''), 3.71 (1H, dd, J =11.4 and 3.9 Hz, H-1), 2.07 (3H, s, Ac), 1.74 (3H, s, H-27), 1.71 (3H, d, J =5.0 Hz, H-6'), 1.68 (3H, s, H-26), 1.16 (3H, d, J =6.9 Hz, H-21), 0.98 (3H, s, H-18 or H-19), and 0.97 (3H, s, H-18 or H-19).

Alkaline Hydrolysis of 4. Compound **4** (5 mg) was hydrolyzed with 3% NaOMe in MeOH at room temperature for 1 h. The reaction mixture was treated as in the case of **2** to yield **3** (1.6 mg), which was identified by the IR and 1H NMR spectra, as well as by a direct TLC comparison. Reversed-phase TLC: R_f 0.68 (MeOH– H_2O , 3:1).

Assay of Cyclic AMP Phosphodiesterase Activity. The phosphodiesterase activity was assayed through a modification of the method of Thompson and Brooker, as described in a previous paper.^{9a)}

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