New Cholestane Glycosides from the Leaves of *Cordyline terminalis*

Akihito Yokosuka,* Takeyuki Suzuki, and Yoshihiro Mimaki*

Laboratory of Medicinal Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences; 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan.

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Four new cholestane glycosides (1—4) were isolated from the leaves of *Cordyline terminalis* (Agavaceae). The structures of the new compounds were determined on the basis of spectroscopic analysis and a few chemical transformations followed by chromatographic and spectroscopic analyses.

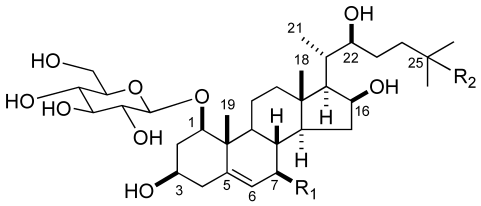
Key words cholestane glycoside; *Cordyline terminalis*; Agavaceae

The genus *Cordyline* (Agavaceae), with about 20 species, is distributed in south-east Asia, Australia, and New Zealand.[1] The occurrence of steroidal sapogenins in several *Cordyline* species such as *C. australis*, *C. neocaledonica*, and *C. cannifolia* has been documented.[2] Previously, we studied the chemical components of the leaves of *C. stricta* and isolated seven new steroidal glycosides together with six known compounds.[3] *C. terminalis* (L.) Kunth is a perennial plant that is found in Malaysia. Although the steroidal sapogenins of smilagenin and sarsasapogenin were isolated from *C. terminalis* var. *petiolaris*,[2] there have been no reports concerning the secondary metabolites of *C. terminalis*. As part of our continuous chemical investigations of plants of the genus *Cordyline*, we have now examined the leaves of *C. terminalis*, resulting in the isolation of four new cholestane glycosides (**1**—**4**). This is a report on the structure elucidation of the new glycosides on the basis of spectroscopic analyses and a few chemical transformations followed by chromatographic and spectroscopic analysis. The cytotoxic activities of the isolated glycosides and aglycones against HL-60 human leukemia cells are also reported.

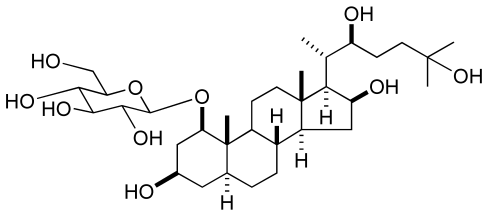
The leaves of *C. terminalis* (6.0 kg) were extracted with MeOH. After removal of solvent, the MeOH extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc. The MeOH eluate-portion was repeatedly subjected to silica gel and octadecylsilanized (ODS) silica gel column chromatography to afford compounds **1**—**4**.

Compound **1** was isolated as an amorphous solid and its molecular formula was deduced to be $C_{33}H_{56}O_{10}$ on the basis of the high resolution (HR)-electrospray ionization (ESI)-time-of-flight (TOF)-MS (*m/z*: 635.3756 $[M+Na]^+$), ^{13}C-NMR, and distortionless enhancement by polarization transfer (DEPT) spectral data. The ^1H-NMR spectrum of **1** contained signals for two tertiary methyl groups at δ 1.26 (3H, s) and 1.24 (3H, s), three secondary methy groups at δ 1.17 (3H, d, J=7.0 Hz) and 0.87 (3H×2, d, J=6.6 Hz), and an olefinic proton at δ 5.89 (1H, brd, J=7.3 Hz) characteristic of a steroidal derivative, and an anomeric proton of a hexopyranosyl group at δ 5.00 (1H, d, J=7.7 Hz). Enzymatic hydrolysis of **1** with naringinase yielded an aglycone (**1a**) and D-glucose. The identification of D-glucose, including the absolute configuration, was carried out by direct HPLC analysis of the hydrolysate using an optical rotation detector. The above data suggested that **1** was a steroidal monoglucoside.

The ^1H-NMR spectrum of **1a** ($C_{27}H_{46}O_5$) contained signals for five exchangeable protons at δ 6.31 (1H, brd, J=6.0 Hz), 6.30 (1H, brs), 6.11 (1H, brd, J=6.0 Hz), 5.88 (1H, brd, J=3.6 Hz), and 5.65 (1H, brd, J=8.3 Hz), which were removed by the addition of HCl vapor, as well as signals for five steroidal methyl groups at δ 1.36 (3H, s), 1.33 (3H, s), 1.23 (3H, d, J=7.0 Hz), and 0.90 (3H×2, d, J=6.5 Hz), and an olefinic proton at δ 5.97 (1H, brd, J=4.5 Hz). Comparison of the ^1H- and ^{13}C-NMR assignments of **1a**, which were established by analysis of the ^1H–^1H shift correlation spectroscopy (COSY), ^1H-detected heteronuclear multiple-quantum coherence (HMQC), and ^1H-detected heteronuclear multiple-bond



	R_1	R_2
1	OH	H
2	H	OH



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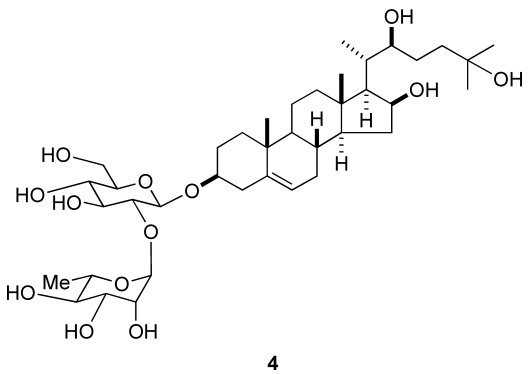

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* To whom correspondence should be addressed. e-mail: yokosuka@toyaku.ac.jp; mimakiy@toyaku.ac.jp

connectivity (HMBC) spectra, with those of the known polyoxysteroid of (22*S*)-cholest-5-ene-1*β*,3*β*,16*β*,22-tetrol,[4] suggested that the structures of the ring A, C, D portions, and side chain (C-1—C-5, C-10—C-27) were identical to those of the reference compound. However, significant differences were recognized in the signals for the ring B portion (C-6—C-9). In the ^{1}H-NMR spectrum of **1a**, signals for five oxymethine protons were observed at δ 4.87 (ddd, J=7.5, 7.0, 3.5 Hz), 4.22 (m), 4.14 (brd, J=6.5 Hz), 4.01 (m, $W_{1/2}$=26.9 Hz), and 3.85 (dd, J=11.0, 4.5 Hz), which were associated with the one-bond coupled carbon signals at δ 71.8, 75.3, 72.2, 67.9, and 78.0, respectively, by the HMQC spectrum. The proton spin-coupling systems revealed by the ^{1}H–^{1}H COSY spectrum, and long-range correlations between δ_H 4.87 and δ_C 43.1 (C-13), δ_H 4.14 and δ_C 140.6 (C-5)/131.4 (C-6), δ_H 4.01 and δ_C 140.6 (C-5), and between δ_H 3.85 and δ_C 49.8 (C-9)/43.2 (C-10) detected in the HMBC spectrum (Fig. 1), allowed the five hydroxy groups to be located at C-1, C-3, C-7, C-16, and C-22. Nuclear Overhauser effect (NOE) correlations between δ_H 4.01 (H-3) and δ_H 3.85 (H-1), δ_H 4.14 (H-7) and δ_H 1.59 (H-9)/1.33 (H-14), and between δ_H 4.87 (H-16) and δ_H 1.71 (H-17) observed in the phase-sensitive nuclear Overhauser effect spectroscopy (NOESY) spectrum (Fig. 2) and the proton spin-coupling constants indicated that **1a** had the usual steroid ring junctions as shown in Fig. 2 and the C-1*β*, C-3*β*, C-7*β*, and C-16*β* configurations. The large J value between H-17 and H-20 (J=11.5 Hz) indicated that the H_{17}–C_{17}–C_{20}–H_{20} part was preferably *trans*-oriented, and an NOE correlation between H-20 and Me-18, Me-21 and H-12eq/H-17, and between H-22 and H-16 made it possible to assign the 17*β* and 20*S* configurations. The absolute configuration at the C-22 chiral center was elucidated as *S* by application of advanced Mosher's method.[5] Compound **1a** was treated with (*S*)- and (*R*)-*α*-methoxy-(trifluoromethyl)phenylacetic acids (MTPA) under the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 4-(dimethylamino)-pyridine (4-DMAP) to yield C-24 (*S*)- and (*R*)-MTPA esters. When the ^{1}H-NMR spectrum of the (*S*)-MTPA ester (**1b**) was compared with that of the (*R*)-MTPA ester (**1c**), the signals assignable to H-16, H-17, Me-18, H-20, and Me-21 of **1b** were observed at lower fields than those of **1c**, whereas the signals for H_2-23, H_2-24, H-25, Me-26, and Me-27 of **1b** appeared at higher fields than those of **1c** (Fig. 3). The structure of **1a** was assigned as (22*S*)-cholest-5-ene-1*β*,3*β*,7*β*,16*β*,22-pentol. The ^{1}H- and ^{13}C-NMR spectra of **1** showed the presence of a *β*-D-glucopyranosyl unit (Glc) [δ_H 5.00 (d, J=7.7 Hz); δ_C 101.3, 75.4, 78.6, 72.3, 78.2, 63.5] in its molecule. An HMBC correlation was observed between H-1 of Glc at δ_H 5.00 and C-1 of the aglycone moiety at δ_C 82.5. Accordingly, the structure of **1** was characterized as (22*S*)-3*β*,7*β*,16*β*,22-tetrahydroxycholest-5-en-1*β*-yl *β*-D-glucopyranoside.

Compound **2** was shown to have a molecular formula of $C_{33}H_{56}O_{10}$ as determined by HR-ESI-TOF-MS analysis (*m/z* 613.3987 $[M+H]^+$). The ^{1}H-NMR spectrum of **2** contained signals for five steroidal methyl groups at δ 1.43 (3H×2, s), 1.29 (3H, s), 1.19 (3H, s), 1.14 (3H, d, J=7.0 Hz), an olefinic proton at δ 5.59 (1H, brd, J=5.5 Hz), and an anomeric proton at δ 5.00 (1H, d, J=7.5 Hz). Enzymatic hydrolysis of **2** with naringinase yielded an aglycone (**2a**; $C_{27}H_{46}O_5$) and D-glucose. The molecular formula of **2a** was the same as that of **1a**. The ^{1}H-NMR spectrum of **2a** contained signals for five exchangeable protons at δ 6.59 (1H, brs), 6.25 (1H, brs), 6.04 (1H, brd, J=5.8 Hz), 5.90 (1H, brs), and 5.80 (1H, brs), which were removed by the addition of HCl vapor. Comparison of the ^{1}H- and ^{13}C-NMR assignments of **2a** with those of **1a** showed that the C-7 oxymethine carbon signal, which was observed at δ 72.2 in **1a**, was displaced by a methylene carbon signal at δ 32.2 in **2a**, and the H_2-7 methylene proton signals at δ 2.02 (1H, m) and δ 1.62 (1H, m) exhibited spin-couplings with the H-6 olefinic proton at δ 5.65 (1H, brd, J=5.4 Hz) and the H-8 methine proton at δ 1.63 (1H, m). On the other hand, the C-25 methine carbon signal, which was observed at δ 28.5 in **1a**, was displaced by a quaternary carbon signal at δ 69.7 in **2a**, and the methyl doublet signals at δ 0.90 (3H×2, d, J=6.5 Hz) in **1a** were observed as singlet methyl signals at δ 1.44 (3H×2, s) in **2a**. The methyl signals at δ 1.44 (3H×2, s) exhibited $^2J_{C-H}$ correlations with the C-25 and $^3J_{C-H}$ correlations with C-24 at δ 42.4 in the HMBC spectrum and were assigned to

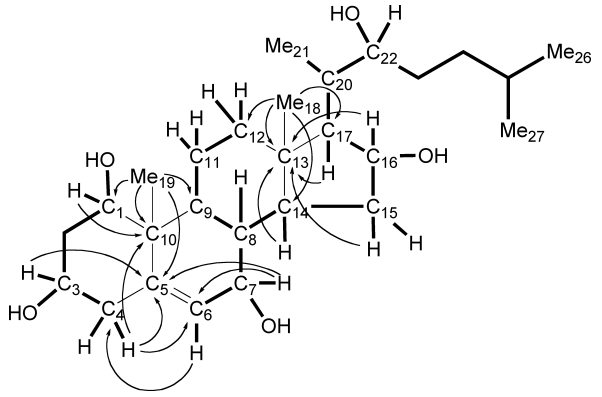


Fig. 1. HMBC Correlations of **1a**

Bold lines indicate the ^{1}H–^{1}H couplings and arrows indicate ^{1}H/^{13}C long-range correlations.

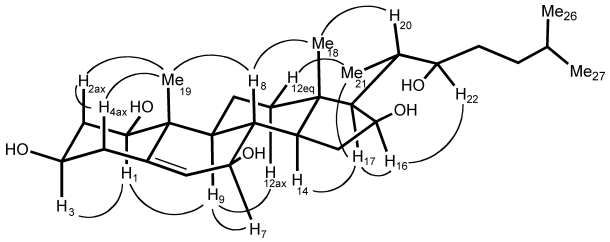
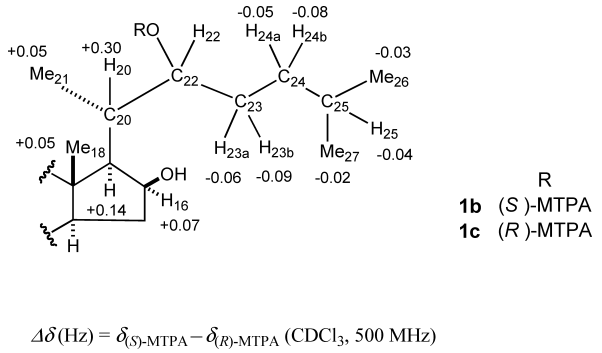


Fig. 2. Important NOE Correlations of **1a**



$\Delta\delta$(Hz) = $\delta_{(S)\text{-MTPA}} - \delta_{(R)\text{-MTPA}}$ ($CDCl_3$, 500 MHz)

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

Me-26 and Me-27. All other signals appeared at almost the same positions between **1a** and **2a**. In the HMBC spectrum of **2**, a long-range correlation was observed between H-1 of Glc at δ 5.00 and C-1 of the aglycone at δ 82.7. Thus, the structure of **2** was determined to be (22*S*)-3β,16β,22,25-tetrahydroxycholest-5-en-1β-yl β-D-glucopyranoside.

Compound **3** was deduced as $C_{33}H_{58}O_{10}$ by HR-ESI-TOF-MS (*m/z*: 637.3908 $[M+Na]^+$). In the ^{1}H-NMR spectrum, the signal due to five steroidal methyl groups [δ_H 1.43 (3H×2, s), 1.13 (3H, s), 1.11 (3H, d, *J*=7.5 Hz), 1.05 (3H, s)] and an anomeric proton [δ_H 5.06 (1H, d, *J*=7.1 Hz)] were observed. Enzymatic hydrolysis of **3** with naringinase yielded an aglycone (**3a**, $C_{27}H_{48}O_5$) and D-glucose. The molecular formula of **3a** was higher than that of **2a** by two hydrogen atoms. The spectral features of **3a** showed close similarity to those of **2a**. Comparison of the ^{1}H-NMR spectrum of **3a** with that of **2a**, the olefinic proton signal due to H-6 was missing in **3a**. Furthermore, the olefinic carbon signals due to C-5 and C-6 in **2a** were displaced by aliphatic carbon signals at δ 43.0 (CH) and 29.1 (CH_2) in **3a**. These data indicated that **3a** was a 5,6-dihydro derivative of **2a**. The ^{13}C-NMR shift of Me-19 at δ 7.6 and the NOE correlation between H-2ax and Me-19 accounted for the A/B *trans* ring junction (H-5α). In the HMBC spectrum of **3**, a long-range correlation was observed between H-1 of Glc at δ 5.06 and C-1 of the aglycone at δ 81.1. On the basis of these data, the structure of **3** was found to be (22*S*)-3β,16β,22,25-tetrahydroxy-5α-cholestan-1β-yl β-D-glucopyranoside.

Compound **4** had the molecular formula $C_{39}H_{66}O_{13}$ by HR-ESI-TOF-MS (*m/z*: 765.4446 $[M+Na]^+$, Calcd for $C_{39}H_{66}NaO_{13}$: 765.4401). The ^{1}H-NMR spectrum contained signals for two anomeric protons at δ 6.39 (1H, brs) and 5.05 (1H, d, *J*=7.5 Hz), as well as five steroidal methyl groups [δ 1.44 (3H×2, s), 1.21 (3H, d, *J*=7.0 Hz), 1.16 (3H, s), and 1.09 (3H, s)] and an olefinic proton [δ 5.33 (1H, d, *J*=4.5 Hz)]. Acid hydrolysis of **4** gave L-rhamnose and D-glucose, while the aglycone decomposed under acidic conditions. On comparison of the ^{13}C-NMR spectrum of **4** with that of **2**, the structures of the ring B—D portions and side chain (C-6—C-27) for the cholestane skeletone are identical to those of **2**. However, differences were recognized in the signals for the ring A portion (C-1—C-5). Analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra of **4** revealed that the aglycone moiety of **4** was (22*S*)-cholest-5-ene-3β,16β,22,25-tetrol and that the sugar moiety of **4** was composed of a terminal α-L-rhamnopyranosyl unit (Rha) [δ_H 6.39 (brs); δ_C 102.0, 72.6, 72.8, 74.2, 69.5, 18.7] and a C-2 substituted β-D-glucopyranosyl unit (Glc) [δ_H 5.05 (d, *J*=7.5 Hz); δ_C 100.4, 77.8, 79.6, 71.8, 78.3, 62.7]. In the HMBC spectrum of **4**, long-range correlations were observed between H-1 of Rha and C-2 of Glc and between H-1 of Glc and C-3 of the aglycone. The structure of **4** was shown to be (22*S*)-16β,22,25-trihydroxycholest-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Compounds **1**—**4** are new cholestane glycosides and their aglycones are new polyhydroxylated cholestane derivatives. Compound **1** is a rare type of cholestane glycoside with a hydroxy group at C-7β, as well as those at the C-1β, C-3β, C-16β, and C-22*S* positions.

The new glycosides (**1**—**4**) and aglycones (**1a**, **2a**) were evaluated for their cytotoxic activities against HL-60 cells. Compound **1a** exhibited weak cytotoxic activity against HL-60 cells with an IC_{50} value of 15.6 μg/mL, while etoposide, used as a positive control, had an IC_{50} value of 0.26 μg/mL. Compounds **1**—**4**, and **2a** were not cytotoxic to HL-60 cells at sample concentrations of 20 μg/mL.

Experimental

Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H-NMR, Karlsruhe, Germany) and a Bruker AV-600 (600 MHz for ^{1}H-NMR) spectrometer using standard Bruker pulse programs. Chemical shifts are given as δ values in reference to tetramethylsilane (TMS) as an internal standard. ESI-TOF-MS data were recorded on a Waters-Micromass LCT mass spectrometer (Manchester, U.K.). Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan), silica gel (Fuji Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on Silica gel 60 F_{254} (thickness: 0.25 mm, Merck, Darmstadt, Germany) and RP_{18} F_{254S} plates (thickness: 0.25 or 0.5 mm, Merck), and spots were visualized by spraying the plates with 10% H_2SO_4 aqueous solution, followed by heating. HPLC was performed with a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 (Tosoh) or a Shodex OR-2 (Showa Denko, Tokyo, Japan) detector, and a Rheodyne injection port. A TSK-gel ODS-100Z column (10 mm i.d.×250 mm, 5 μm, Tosoh) was employed for preparative HPLC. The following materials and reagents were used for cell culture assay: 96-well flat-bottom plate (Iwaki Glass, Chiba, Japan); RPMI 1640 medium, etoposide, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, U.S.A.); fetal bovine serum (FBS) (BioWhittaker, Walkersville, MO, U.S.A.); penicillin G sodium salt and streptomycin sulfate (Gibco, Grand Island, NY, U.S.A.). All other chemicals used were of biochemical reagent grade.

Plant Material *C. terminalis* was purchased from Fuji Green (Tokyo, Japan) in September 2006. A voucher specimen has been deposited in our laboratory (voucher No. CT-2006-001, Laboratory of Medicinal Pharmacognosy).

Extraciton and Isolation The leaves of *C. terminalis* (fresh weight, 6.0 kg) were extracted with MeOH (45 L). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (420 g) was passed through a Diaion HP-20 column, successively eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc. Column chromatography (CC) of the MeOH-eluate portion (55 g) on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (9 : 1 : 0, 40 : 10 : 1, 30 : 10 : 1, 20 : 10 : 1, 1 : 1 : 0), and finally with MeOH alone, gave 6 fractions (I—VI). Fraction IV was chromatographed on ODS silica gel eluted with MeCN–H_2O (2 : 5) to give 9 subfractions (IVa—IVi). Fraction IVa was further separated by ODS silica gel CC eluted with MeOH–H_2O (3 : 2), silica gel CC with $CHCl_3$–MeOH (4 : 1, 3 : 1), and preparative HPLC using MeOH–H_2O (3 : 1) to yield **1** (67.0 mg) and **2** (24.8 mg). Fraction IVe was chromatographed on ODS silica gel eluted with MeOH–H_2O (1 : 1) to give 11 subfractions (IVea—IVek). Fraction IVej was separated by silica gel CC eluted with $CHCl_3$–MeOH (5 : 1) to yield **3** (30.5 mg). Fraction IVg was separated by ODS silica gel CC eluted with

Table 1. ^{13}C-NMR Data for **1**, **1a**, **2**, **2a**, **3**, **3a**, and **4** in C_5D_5N

	1	1a	2	2a	3	3a	4
1	82.5	78.0	82.7	78.2	81.1	77.3	37.5
2	37.5	43.8	37.5	43.5	37.5	44.0	30.2
3	67.8	67.9	67.8	68.1	67.7	67.9	75.0
4	43.2	43.0	43.7	44.0	39.6	39.6	39.0
5	139.7	140.6	139.6	140.3	43.0	43.0	140.9
6	131.5	131.4	124.8	124.5	29.0	29.1	121.9
7	71.7	72.2	31.9	32.2	32.3	32.5	32.2
8	41.8	41.8	33.3	33.2	36.6	36.4	31.9
9	48.7	49.8	50.4	51.2	54.9	55.8	50.5
10	42.5	43.2	42.8	42.3	41.4	42.0	37.0
11	23.9	24.3	23.8	24.2	23.7	24.7	21.1
12	40.9	41.2	40.9	41.1	41.1	41.4	40.4
13	43.1	43.1	42.4	42.4	42.5	42.6	42.7
14	55.1	55.3	54.9	55.1	54.8	55.0	54.9
15	40.2	40.3	37.2	37.2	37.2	37.3	37.0
16	71.8	71.8	71.5	71.5	71.5	71.5	71.6
17	57.7	57.8	58.3	58.3	58.4	58.4	58.1
18	13.8	13.8	13.7	13.6	13.8	13.8	13.4
19	14.4	13.5	14.7	14.2	8.2	7.6	19.4
20	36.1	36.1	36.1	36.1	36.1	36.1	36.0
21	15.3	15.3	15.6	15.6	15.6	15.6	15.6
22	75.3	75.3	76.4	76.3	76.4	76.4	76.3
23	32.1	32.2	28.9	28.9	28.9	28.9	28.9
24	36.7	36.8	42.3	42.4	42.2	42.3	42.3
25	28.4	28.5	69.7	69.7	69.7	69.7	69.7
26	22.7	22.8	29.7	29.7	29.7	29.8	29.7
27	23.0	23.0	30.5	30.5	30.5	30.5	30.5
1′	101.3		101.2		100.4		100.4
2′	75.4		75.4		75.5		77.8
3′	78.6		78.6		78.6		79.6
4′	72.3		72.4		72.2		71.8
5′	78.2		78.2		78.3		78.3
6′	63.5		63.6		63.5		62.7
1″							102.0
2″							72.6
3″							72.8
4″							74.2
5″							69.5
6″							18.7

MeOH–H_2O (3 : 2), silica gel CC with $CHCl_3$–MeOH (3 : 1), and preparative HPLC using MeCN–H_2O (10 : 23) to yield **4** (5.9 mg).

Compound **1**: Amorphous solid. $[\alpha]_D^{25}$ −7.8 (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 635.3756 $[M+Na]^+$ (Calcd for $C_{33}H_{56}NaO_{10}$: 635.3771). IR ν_{max} (film) cm^{-1}: 3215 (OH), 2951 (CH), 1170 and 1073 (C–O). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 5.89 (1H, brd, J=7.3 Hz, H-6), 5.00 (1H, d, J=7.7 Hz, Glc-1), 4.81 (1H, m, H-16), 4.55 (1H, dd, J=11.4, 2.7 Hz, Glc-6a), 4.34 (1H, dd, J=11.4, 5.8 Hz, Glc-6b), 4.23 (1H, dd, J=8.5,8.5 Hz, Glc-3), 4.19 (1H, m, H-22), 4.13 (1H, dd, J=8.5, 8.5 Hz, Glc-4), 4.05 (1H, dd, J=8.5, 7.7 Hz, Glc-2), 4.04 (1H, brd, J=8.0 Hz, H-7), 4.00 (1H, dd, J=11.4, 4.0 Hz, H-1), 3.94 (1H, m, Glc-5), 3.89 (1H, m, $W_{1/2}$=20.8 Hz, H-3), 2.59 (1H, m, H-20), 1.69 (1H, m, H-17), 1.26 (3H, s, Me-19), 1.24 (3H, s, Me-18), 1.17 (3H, d, J=7.0 Hz, Me-21), 0.87 (3H×2, d, J=6.6 Hz, Me-26, Me-27). ^{13}C-NMR (125 MHz, C_5D_5N): see Table 1.

Enzymatic Hydrolysis of 1 Compound **1** (35.0 mg) was treated with naringinase (Sigma Aldrich, EC 232-962-4, 90.3 mg) in AcOH/AcOK buffer (pH 4.3, 2 mL) at room temperature for 72 h. The reaction mixture was subjected to silica gel CC eluted with $CHCl_3$–MeOH (19 : 1) to yield **1a** (17.2 mg) and a sugar fraction (10.0 mg), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2, UG80 (4.6 mm i.d×250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN H_2O (85 : 15); flow rate, 1.0 mL/min; detection, OR. Identification of D-glucose present in the sugar fraction was carried out by comparison of its retention time and optical rotation with those of an authentic sample. t_R (min): 13.5 (D-glucose, positive optical rotation).

Compound **1a**: Amorphous solid. $[\alpha]_D^{25}$ −9.3 (c=0.07, MeOH). HR-ESI-TOF-MS m/z: 473.3246 $[M+Na]^+$ (Calcd for $C_{27}H_{46}NaO_5$: 473.3243). IR ν_{max} (film) cm^{-1}: 3293 (OH), 2955 (CH), 1097 (C–O). ^{1}H-NMR (600 MHz, C_5D_5N) δ: 5.97 (1H, brd, J=4.5 Hz, H-6), 4.87 (1H, ddd, J=7.5, 7.0, 3.5 Hz, H-16), 4.22 (1H, m, H-22), 4.14 (1H, brd, J=6.5 Hz, H-7), 4.01 (1H, m, $W_{1/2}$=26.9 Hz, H-3), 3.85 (1H, dd, J=11.0, 4.5 Hz, H-1), 2.64 (1H, m, H-20), 1.71 (1H, dd, J=11.5, 7.0 Hz, H-17), 1.36 (3H, s, Me-19), 1.33 (3H, s, Me-18), 1.23 (3H, d, J=7.0 Hz, Me-21), 0.90 (3H×2, d, J=6.5 Hz, Me-26, 27). ^{13}C-NMR (150 MHz, C_5D_5N): see Table 1.

Preparation of 1b and 1c To a solution of **1a** (4.0 mg) in CH_2Cl_2 (0.4 mL), (*R*)-(−)-MTPA (21.3 mg), EDC·HCl (13.8 mg), and 4-DMAP (3.8 mg) were added, and the mixture was stirred at room temperature for 48 h. The reaction mixture was poured into optimal H_2O, which was extracted with EtOAc (3 mL×3). Evaporation of the solution followed by silica gel CC eluted with hexane–acetone (4 : 1) gave **1b** (8.4 mg). Using the same procedure, a mixture of **1a** (4.0 mg) and (*S*)-MTPA (21.4 mg) yielded **1b** (7.7 mg).

Compound **2**: Amorphous solid. $[\alpha]_D^{25}$ −39.3 (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 613.3987 $[M+H]^+$ (Calcd for $C_{33}H_{57}O_{10}$: 613.3952). IR ν_{max} (film) cm^{-1}: 3224 (OH), 2940 (CH), 1158 and 1075 (C–O). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 5.59 (1H, brd, J=5.5 Hz, H-6), 5.00 (1H, d, J=7.5 Hz, Glc-1), 4.74 (1H, ddd, J=7.7, 7.0, 3.5 Hz, H-16), 4.56 (1H, brd, J=11.7 Hz, Glc-6a), 4.35 (1H, dd, J=11.7, 4.2 Hz, Glc-6b), 4.24 (1H, dd, J=8.8, 8.8 Hz, Glc-3), 4.19 (1H, m, H-22), 4.15 (1H, dd, J=8.8, 8.8 Hz, Glc-4), 4.07 (1H, dd, J=8.8, 7.5 Hz, Glc-2), 4.01 (1H, dd, J=11.6, 4.0 Hz, H-1), 3.89 (1H, m, $W_{1/2}$=20.8 Hz, H-3), 2.62 (1H, m, H-20), 1.62 (1H, dd, J=11.0, 7.0 Hz, H-17), 1.43 (3H×2, s, Me-26, 27), 1.29 (3H, s, Me-19), 1.19 (3H, s, Me-18), 1.14 (3H, d, J=7.0 Hz, Me-21). ^{13}C-NMR (125 MHz, C_5D_5N): see Table 1.

Enzymatic Hydrolysis of 2 Compound **2** (1.7 mg) was treated with naringinase (20.4 mg) in AcOH/AcOK buffer (pH 4.3, 1 mL) at room temperature for 168 h. The reaction mixture was subjected to silica gel CC eluted with $CHCl_3$–MeOH (9 : 1) to yield **2a** (0.7 mg) and a sugar fraction (0.4 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose t_R (min): 13.7 (D-glucose, positive optical rotation).

Compound **2a**: Amorphous solid. $[\alpha]_D^{25}$ −14.9 (c=0.07, MeOH). HR-ESI-TOF-MS m/z: 473.3252 $[M+Na]^+$ (Calcd for $C_{27}H_{46}NaO_5$: 473.3243). IR ν_{max} (film) cm^{-1}: 3364 (OH), 2961 and 2923 (CH), 1095 (C–O). ^{1}H-NMR (600 MHz, C_5D_5N) δ: 5.65 (1H, brd, J=5.4 Hz, H-6), 4.79 (1H, m, H-16), 4.21 (1H, m, H-22), 4.00 (1H, m, $W_{1/2}$=23.5 Hz, H-3), 3.85 (1H, dd, J=10.5, 6.0 Hz, H-1), 2.66 (1H, m, H-20), 2.02 (1H, m, H-7a),

1.65 (1H, dd, J=12.0, 5.5 Hz, H-17), 1.63 (1H, m, H-8), 1.62 (1H, m, H-7b), 1.44 (3H×2, s, Me-26, 27), 1.39 (3H, s, Me-19), 1.26 (3H, s, Me-18), 1.20 (3H, d, J=7.5 Hz, Me-21). ^{13}C-NMR (150 MHz, C_5D_5N): see Table 1.

Compound **3**: Amorphous solid. $[\alpha]_D^{25}$ −42.4 (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 637.3908 $[M+Na]^+$ (Calcd for $C_{33}H_{58}NaO_{10}$: 637.3928). IR ν_{max} (film) cm^{-1}: 3282 (OH), 2931 (CH), 1161 and 1076 (C-O). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 5.06 (1H, d, J=7.1 Hz, Glc-1), 4.77 (1H, ddd, J=7.9, 7.0, 4.8 Hz, H-16), 4.58 (1H, dd, J=11.0, 2.0 Hz, Glc-6a), 4.35 (1H, dd, J=11.0, 6.0 Hz, Glc-6b), 4.23 (1H, dd, J=9.0, 9.0 Hz, Glc-3), 4.17 (1H, m, H-22), 4.14 (1H, dd, J=9.0, 9.0 Hz, Glc-4), 4.06 (1H, dd, J=9.0, 7.1 Hz, Glc-2), 4.02 (1H, dd, J=11.5, 4.0 Hz, H-1), 3.89 (1H, m, $W_{1/2}$=22.0 Hz, H-3), 2.61 (1H, m, H-20), 1.59 (1H, dd, J=10.0, 7.0 Hz, H-17), 1.43 (3H×2, s, Me-26, 27), 1.13 (3H, s, Me-19), 1.11 (3H, d, J=7.5 Hz, Me-21), 1.05 (3H, s, Me-18). ^{13}C-NMR (125 MHz, C_5D_5N): see Table 1.

Enzymatic Hydrolysis of 3 Compound **3** (5.1 mg) was treated with naringinase (40.0 mg) in AcOH/AcOK buffer (pH 4.3, 2 mL) at room temperature for 168 h. The reaction mixture was subjected to silica gel CC eluted with $CHCl_3$–MeOH (9 : 1) to yield **3a** (2.3 mg) and a sugar fraction (1.0 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose t_R (min): 14.4 (D-glucose, positive optical rotation).

Compound **3a**: Amorphous solid. $[\alpha]_D^{25}$ −11.5 (c=0.115, MeOH). HR-ESI-TOF-MS m/z: 453.3585 $[M+H]^+$ (Calcd for $C_{27}H_{49}O_5$: 453.3580). IR ν_{max} (film) cm^{-1}: 3347 (OH), 2962 and 2926 (CH), 1093 (C-O). ^{1}H-NMR (600 MHz, C_5D_5N) δ: 4.79 (1H, m, H-16), 4.21 (1H, m, H-22), 4.00 (1H, m, $W_{1/2}$=22.0 Hz, H-3), 3.75 (1H, dd, J=10.5, 5.0 Hz, H-1), 2.67 (1H, m, H-20), 1.63 (1H, dd, J=10.5, 7.0 Hz, H-17), 1.44 (3H×2, s, Me-26, 27), 1.23 (3H, s, Me-18), 1.17 (3H, s, Me-19), 1.20 (3H, d, J=7.0 Hz, Me-21). ^{13}C-NMR (150 MHz, C_5D_5N) δ: see Table 1.

Compound **4**: Amorphous solid. $[\alpha]_D^{25}$ −65.6 (c=0.10, MeOH). HR-ESI-TOF-MS m/z: 765.4446 $[M+Na]^+$ (Calcd for $C_{39}H_{66}NaO_{13}$: 765.4401). IR ν_{max} (film) cm^{-1}: 3422 and 3399 (OH), 2935 (CH), 1127 and 1069 (C–O). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 6.39 (1H, br s, Rha-1), 5.33 (1H, br d, J=4.5 Hz, H-6), 5.05 (1H, d, J=7.5 Hz, Glc-1), 5.00 (1H, dq, J=9.0, 6.0 Hz, Rha-5), 4.79 (1H, ddd, J=7.0, 7.0, 3.5 Hz, H-16), 4.81 (1H, br d, J=3.0 Hz, Rha-2), 4.64 (1H, dd, J=9.0, 3.0 Hz, Rha-3), 4.53 (1H, dd, J=11.5, 2.0 Hz, Glc-6a), 4.37 (1H, dd, J=11.5, 4.0 Hz, Glc-6b), 4.33 (1H, dd, J=9.0, 9.0 Hz, Rha-4), 4.29 (1H, dd, J=9.0, 7.5 Hz, Glc-2), 4.22 (1H, m, H-22), 4.28 (1H, dd, J=9.0, 9.0 Hz, Glc-3), 4.18 (1H, dd, J=9.0, 9.0 Hz, Glc-4), 3.95 (1H, m, $W_{1/2}$=22.5 Hz, H-3), 2.65 (1H, m, H-20), 1.79 (3H, d, J=6.0 Hz, Rha-6), 1.64 (1H, dd, J=11.0, 7.0 Hz, H-17), 1.44 (3H×2, s, Me-26, 27), 1.21 (3H, d, J=7.0 Hz, Me-21), 1.16 (3H, s, Me-18), 1.09 (3H, s, Me-19). ^{13}C-NMR (125 MHz, C_5D_5N): see Table 1.

Acid Hydrolysis of 4 A solution of **4** (3.9 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 mL) was heated at 95°C for 2.5 h under Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-96SB (Organo, Tokyo, Japan). The neutralized mixture was subjected to silica gel CC eluted with hexane–Me_2CO (19 : 1) to yield a sugar fraction (1.3 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose and L-rhamnose t_R (min): 13.7 (D-glucose, positive optical rotation), 7.4 (L-rhamnose, negative optical rotation).

HL-60 Cell Culture and Assay The cell growth was measured with a modified MTT reduction assay as described in a previous paper.[6] Briefly, HL-60 cells were maintained in RPMI 1640 medium containing heat-inactivated 10% (v/v) FBS supplemented with L-glutamine, 100 unit/mL penicillin G sodium salt, and 100 μg/mL streptomycin sulfate. The cells (4×10^4 cells/mL) were continuously treated with each compound for 72 h, and cell growth was measured with an MTT reduction assay procedure. A dose–response curve was plotted for **1a** and the concentration giving 50% inhibition (IC_{50}) was calculated.

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