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PHYTOCHEMISTRY

Cholestane rhamnosides from the bulbs of *Ornithogalum saundersiae*

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Abstract

Phytochemical examination of the bulbs of *Ornithogalum saundersiae* yielded six cholestane rhamnosides, two of which had previously been isolated from the same plant material. However, detailed spectroscopic analysis of the aglycone led us to revise the configuration of the C-11 hydroxyl group of the latter two and reassign their structures as (22*S*)-cholest-5-ene-3β,11α,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside and (22*S*)-cholesta-5,24-diene-3β,11α,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside, respectively. The other four are new naturally occurring constituents and their structures were determined to be (22*S*)-cholest-5-ene-3β,11α,16β,22-tetrol 16-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside), (22*S*)-cholest-5-ene-3β,11α,16β,22-tetrol 16-*O*-{2-*O*-acetyl-3-*O*-(3,4,5-trimethoxybenzoyl)-α-L-rhamnopyranoside}, (22*S*)-cholest-5-ene-3β,11α,16β,22-tetrol 16-*O*-{2-*O*-acetyl-3-*O*-(*p*-methoxybenzoyl)-α-L-rhamnopyranoside} and (22*S*)-cholesta-5,24-diene-3β,11α,16β,22-tetrol 16-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside), respectively. The isolated compounds were evaluated for their cytostatic activity against leukemia HL-60 cells.

Keywords: *Ornithogalum saundersiae*; Liliaceae; Bulbs; Cholestane rhamnosides; Cytostatic activity; HL-60 cells

1. Introduction

During our systematic studies on the chemical constituents of plants of the family Liliaceae and their biological activities, we have disclosed that the bulbs of *Ornithogalum saundersiae* contain abundant cholestane glycosides (Kubo et al., 1992b; Kuroda, Mimaki & Sashida, 1999; Kuroda, Mimaki, Sashida, Nikaido & Ohmoto, 1993; Kuroda et al., 1995, 1997; Mimaki et al., 1996a,b,c, 1997), some of which are unique in structures having a rearranged cholestane skeleton (Kuroda et al., 1993, 1997, submitted for publication; Mimaki et al., 1996a,c) and exhibit potent cytostatic activity against malignant tumor cells (Kuroda et al., 1997, 1999; Mimaki et al., 1996a,b,c, 1997). Further analysis of the bulbs of *O. saundersiae* has resulted in the isolation of six cholestane glycosides (**1**–**6**). We now report the structural revision of **1** and **5**, which had previously been isolated from this plant (Kubo, Mimaki, Sashida, Nikaido & Ohmoto, 1992a) and structural determination of four new cholestane rhamnosides (**2**–**4** and **6**) on the basis of spectroscopic analysis, including two-dimensional NMR techniques and the results of hydrolysis. The activity of **1**–**6** against leukemia HL-60 cells was also investigated.

2. Results and discussion

Compounds **1**–**6** were purified as described in Section 3.

The physical properties and spectral data of **1** and **5** completely agreed with those of (22*S*)-cholest-5-ene-3β,11β,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside and (22*S*)-cholesta-5,24-diene-3β,11β,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside, which had previously been isolated from the same plant source (Kubo et al., 1992a).

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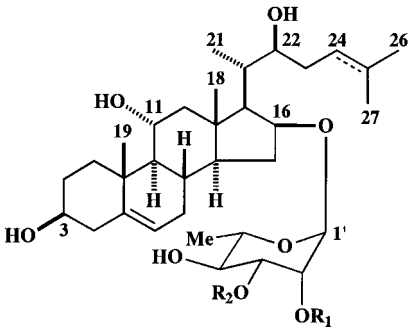
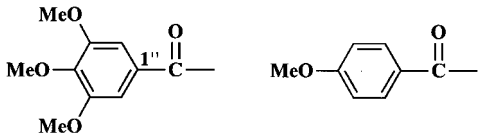


	R_1	R_2	
1	H	H	
2	Ac	Ac	
3	Ac	3,4,5-trimethoxybenzoyl	
4	Ac	*p*-methoxybenzoyl	
5	H	H	Δ^{24}
6	Ac	Ac	Δ^{24}



3,4,5-trimethoxybenzoyl *p*-methoxybenzoyl

However, detailed analysis of the aglycone using extensive spectroscopic methods led us to revise the configuration of the C-11 hydroxyl group. Acid hydrolysis of **1** with 1 M HCl in dioxane–H_2O gave an aglycone (**1a**) and L-rhamnose. The complete assignments of the ^1H and ^{13}C NMR signals of **1a** were carried out by the concerted use of the ^1H-^1H COSY, HMQC and HMBC spectra as shown in . This NMR information showed the presence of the C-3, C-11, C-16 and C-22 hydroxyl groups on a cholestane skeleton. The configuration of the C-11 hydroxyl group was previously assigned as an 11β(ax) by the conventional comparison of the ^1H NMR data with those of cholesterol. In the high-resolution ^1H NMR spectrum (500 MHz) of **1a** measured in pyridine-d_5, to which was added the vapor of HCl to remove the signals due to the exchangeable protons, the signal due to H-11 was observed as a ddd multiplicity at δ 4.43 ($^3J_{H\text{-}11,H\text{-}9}=10.7$ Hz, $^3J_{H\text{-}11,H\text{-}12ax}=10.7$ Hz and $^3J_{H\text{-}11,H\text{-}12eq}=4.7$ Hz). This unequivocally indicated an α(eq)-orientation of the C-11 hydroxyl group, which was strongly supported by NOE correlations from H-11 to both Me-18 and Me-19. The significant downfield shift of the H-1β(eq) proton [δ 3.36 (ddd, J = 13.9, 3.3, 3.3 Hz)] was considered to be caused by the interaction with the 11α-hydroxyl group. The 3β, 16β and 22*S* configurations, and the usual steroidal ring and side chain junctions, B/C *trans* and C/D *trans*, and 17β-

Table 1
^1H and ^{13}C NMR spectral data for compound **1a** in C_5D_5N

Position	^1H	J (Hz)	^{13}C
1 ax	1.57		40.0
1 eq	3.36 ddd	13.9, 3.3, 3.3	
2 ax	2.03		32.9
2 eq	2.19 br dd	12.7, 2.1	
3	3.97 m	21.1[a]	71.7
4 ax	2.77		44.2
4 eq	2.75		
5	–		142.9
6	5.56 br d	5.4	121.0
7 α	1.74		32.4
7 β	2.07		
8	1.72		32.1
9	1.42 dd	10.7, 10.7	57.3
10	–		38.9
11	4.43 ddd	10.7, 10.7, 4.7	68.2
12 ax	1.67		52.4
12 eq	2.70 dd	12.0, 4.7	
13	–		43.4
14	1.19		54.6
15 α	2.37 ddd	12.7, 7.7, 7.7	37.2
15 β	1.58		
16	4.84 ddd	7.7, 7.2, 4.5	71.8
17	1.80 dd	11.1, 7.2	58.1
18	1.31 s		14.7
19	1.47 s		19.3
20	2.64		36.0
21	1.26 d	7.1	15.1
22	4.20 br d	8.8	75.1
23 a	1.83		31.9
23 b	1.71		
24 a	1.76		36.7
24 b	1.39		
25	1.63		28.4
26	0.93 d	6.5	22.8
27	0.92 d	6.6	23.0

[a] $W_{1/2}$.

orientation in **1a** were ascertained by the ^1H and ^{13}C NMR data (Table 1), including NOE information (Fig. 1), and by the application of the modified Mosher's method (Ohtani, Kusumi, Kashman & Kakisawa, 1991) (Fig. 2). Furthermore, we inquired into the stereochemistry at C-20, which was conventionally assigned as *S* in the previous report. The stereochemistry was examined by using molecular modeling, $^3J_{H,H}$ value and NOESY data. A combination of molecular mechanics (MM) and molecular dynamics (MD) calculations in the MM2 force field as implemented in MacroModel 6.0 was performed on two possible compounds with C-20*S* and C-20*R* configurations. The H_{17}–C_{17}–C_{20}–H_{20} torsion angle was almost identical between the minimum energy conformers of C-20*S* and C-20*R* models; −176.4° for C-20*S* and −172.4° for C-20*R* (Fig. 3). The experimental J value (11.1 Hz) of H-17/H-20 almost corresponded to that (20*S*: 12.8 Hz; 20*R*: 12.6 Hz) calculated through

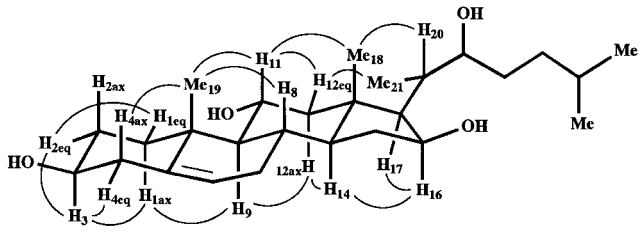


Fig. 1. NOE correlations of **1a**.

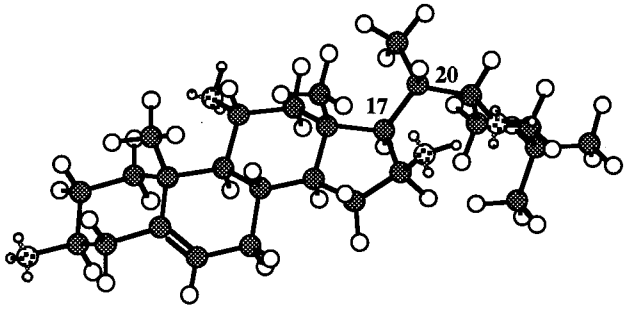


Fig. 3. The lowest energy conformer of **1a**.

the application of the given dihedral angle to the advanced Karplus-type equation proposed by Haasnoot, De Leeuw and Altona (1980). Since it was indicated by the above data that H-20 lay toward Me-18 and that the C-17/C-20 bond was frozen (Nes et al., 1998), NOE correlations observed between Me-18 and H-20, and between H-12β(eq) and Me-21 made it possible to assign the C-20 configuration as *S*. The evidence presented above led us to revise the structure of **1a** to (22*S*)-cholest-5-ene-3β,11α,16β,22-tetrol and, consequently, **1** to (22*S*)-cholest-5-ene-3β,11α,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside. Compound **5** was correlated to **1** by hydrogenation (Kubo et al., 1992a) and has also been reassigned as (22*S*)-cholesta-5,24-diene-3β,11α,16β,22-tetrol 16-*O*-α-L-rhamnopyranoside.

Compound **2**, isolated as an amorphous solid, $[\alpha]_D$ −28.0° (MeOH) showed in the HRFABMS (positive mode) an accurate $[M+Na]^+$ ion at *m/z* 687.4048 in accordance with the empirical molecular formula $C_{37}H_{60}O_{10}$, also deduced on the basis of ^{13}C NMR data. The ^1H NMR spectrum of **2** showed signals for five steroid methyl protons at δ 1.41 (3H, s), 1.22 (3H, d, *J* = 6.9 Hz), 1.08 (3H, d, *J* = 6.5 Hz), 1.02 (3H, d, *J* = 6.5 Hz) and 0.99 (3H, s), an olefinic proton at δ 5.50 (1H, br d, *J* = 5.2 Hz) and an anomeric proton at δ 5.02 (1H, d, *J* = 1.3 Hz). The above ^1H NMR features and ^{13}C NMR spectral data were essentially analogous to those of **1**. The presence of two acetyl groups in the molecule was shown by the IR (ν_{max} 1745 and 1725 cm^{-1}), ^1H NMR [δ 2.10 and 2.00 (each 3H, s)] and ^{13}C NMR [δ 170.4 and 170.1 (C=O), and 20.8 and 20.7 (Me)] spectra. Treatment of **2** with 3% NaOMe in MeOH gave **1**. Therefore, **2** was a diacetate of **1**. In the ^1H NMR spectrum of **2**, the signals assignable to H-2 and H-3 of the rhamnose moiety were moved downfield by 1.17 and 1.48 ppm in comparison with those of **1** and observed at δ 5.64 (dd, *J* = 3.3, 1.3 Hz) and 5.78 (dd, *J* = 9.9, 3.3 Hz), respectively. Thus, the acetyl groups were linked to the rhamnose C-2 and C-3 positions and the structure was formulated as (22*S*)-cholest-5-ene-3β,11α,16β,22-tetrol 16-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside).

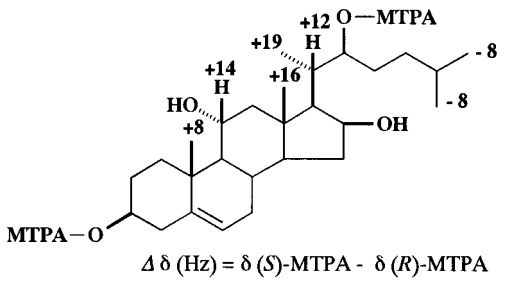


Fig. 2. Chemical shift differences between (*S*)-MPTA ester (**1c**) of **1a** and (*R*)-MPTA ester (**1b**) of **1a**.

The ^1H and ^{13}C NMR signals arising from the aglycone and rhamnosyl moieties of compound **3** ($C_{45}H_{68}O_{13}$) were almost identical to those of **2**. The existence of a 3,4,5-trimethoxybenzoyl group as well as an acetyl group in **3** was indicated by the IR (ν_{max} 1745 and 1720 cm^{-1}), UV [λ_{max} 266 (log ε 4.02)], ^1H NMR [δ 7.58 (2H, s), 3.89 (3H, s) and 3.73 (3H × 2, s); δ 2.18 (3H, s)] and ^{13}C NMR [δ 165.9 (C=O), 153.6 (C × 2), 143.0 (C), 125.9 (C), 107.7 (CH × 2), 60.6 (OMe) and 56.0 (OMe × 2); 170.0 (C=O) and 20.8 (Me)] spectra. Alkaline methanolysis of **3** with 3% NaOMe in MeOH furnished methyl 3,4,5-trimethoxybenzoate and **1**. The downfield shifts by *O*-acylation could be detected at H-2 and H-3 of the rhamnosyl moiety, leading to the two possible structures with regard to the linkage positions of the two acyl groups, that is, 2-*O*-acetyl-3-*O*-(3,4,5-trimethoxybenzoyl)-α-L-rhamnosyl or 2-*O*-(3,4,5-trimethoxybenzoyl)-3-*O*-acetyl-α-L-rhamnosyl. This was solved by analysis of the HMBC spectrum. A carbonyl carbon signal at δ 170.0 showed long-range correlations with the proton signals due to the acetyl methyl group and H-2 of rhamnosyl moiety at δ 5.82 (dd, *J* = 3.3, 1.4 Hz). Another carbonyl carbon at δ 165.9 was correlated to the aromatic protons and H-3 proton of the rhamnosyl at δ 6.09 (dd, *J* = 9.7, 3.3 Hz). From the evidence presented above, the acetyl and 3,4,5-trimethoxybenzoyl groups were shown to be located at C-2 and C-3 of the rhamnosyl moiety, respectively, and the structure of **3** was elucidated as (22*S*)-cholest-5-ene-

3β,11α,16β,22-tetrol 16-*O*-{2-*O*-acetyl-3-*O*-(3,4,5-trimethoxybenzoyl)-α-L-rhamnopyranoside}.

The ^{1}H and ^{13}C NMR spectra of compound **4** ($C_{43}H_{64}O_{11}$) were completely superimposable on those of **3**, except for the aromatic region signals due to the substituted benzoyl moiety. The aromatic acid linked to C-3 of the rhamnosyl residue was suggested to be *p*-methoxybenzoic acid by the UV [λ_{max} 260 (log ε 3.68)], ^{1}H NMR [δ 8.21 and 6.96 (each 2H, d, J = 8.7 Hz), and 3.66 (3H, s)] and ^{13}C NMR [δ 165.9 (C=O), 163.8 (C), 132.0 (C × 2), 123.5 (C), 114.1 (CH × 2) and 55.4 (OMe)] spectra. Alkaline hydrolysis of **4** gave *p*-methoxybenzoic acid and **1**. The structure of **4** was shown to be (22*S*)-cholest-5-ene-3β,11α,16β,22-tetrol 16-*O*-{2-*O*-acetyl-3-*O*-(*p*-methoxybenzoyl)-α-L-rhamnopyranoside}.

Spectral properties of compound **6** ($C_{37}H_{58}O_{10}$) were closely related to those of **2** and indicative of it being a cholestane rhamnoside with two acetyl groups. The ^{1}H NMR spectrum of **6** showed two three-proton singlet signals at δ 1.79 and 1.74 assignable to methyl groups on a double bond, which were present as three-proton doublet signals at δ 1.08 (J = 6.5 Hz) and 1.02 (J = 6.5 Hz) attributable to the Me-26 and Me-27 groups in **2**. All other signals were almost superimposable between these two compounds. Alkaline methanolysis of **6** gave **5**. The above-mentioned data indicated that **6** was the corresponding 24-ene derivative of **2**, and that the structure was (22*S*)-cholesta-5,24-diene-3β,11α,16β,22-tetrol 16-*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranoside).

The cytostatic activity of **1**–**6** on human promyelocytic leukemia HL-60 cells was evaluated as in the preceding paper (Kuroda et al., 1999). Compounds **1**, **2**, **3**, **4** and **6** exhibited potent cytostatic activity with the GI_{50} values of 0.19, 6.9, 1.8, 0.022 and 0.80 μM, respectively. Subsequent evaluation of **1**, the main constituent of the plant, in the National Cancer Institute 60 cell line assay (Monks et al., 1991) showed that the mean concentrations required to achieve GI_{50}, TGI and LC_{50} levels against the panel of cells tested were 1.5, 20 and 69 μM, respectively. Compound **1** displayed a specific activity towards the leukemia cell lines (mean GI_{50} 0.052 μM; mean TGI 0.23 μM; mean LC_{50} 27 μM). Furthermore, some cell lines were sensitive to it. These cell lines included the colon cancer KM12 (GI_{50} 0.41 μM; TGI 2.5 μM; LC_{50} 14 μM), CNS cancer SF-539 (GI_{50} 0.015 μM; TGI 0.24 μM; LC_{50} 4.6 μM), melanoma SK-MEL-28 (GI_{50} 0.22 μM; TGI 2.6 μM; LC_{50} 8.5 μM) and breast cancer MCF7 (GI_{50} 0.022 μM; TGI 0.37 μM; LC_{50} 10 μM) (Table 3). The acute toxicity assay on **1** has been done and the maximum tolerated dose in mice was determined to be 400 mg/kg. The in vivo antitumor testing for **1** is in progress.

3. Experimental

3.1. General

As for preceding paper, except, in addition, NMR experiments used Bruker DPX-400 (400 MHz for ^{1}H NMR), Bruker DRX-500 (500 MHz for ^{1}H NMR spectrometers). HPLC also employed a Capcell Pak C_{18} column (Shiseido, Japan, 4.6 mm i.d. × 250 mm, ODS, 5 μm) for analytical HPLC. Cell culture and assay for cytostatic activity: microplate reader, Inter Med Immuno-Mini NJ-2300 (Japan); 96-well flat-bottom plate, Iwaki Glass (Japan); HL-60 cells, ICN Biomedicals (USA); RPMI 1640 medium, Gibco BRL (USA); MTT, Sigma (USA).

3.2. Plant material

See preceding paper Kuroda et al., 1999.

3.3. Extraction and isolation

As for preceding paper, fraction I was subjected to ODS silica gel CC eluting with MeOH–H_2O (17:3) and divided into five additional fractions (Ia–Ie). Fraction Ia was subjected to silica gel CC eluting with $CHCl_3$–MeOH (10:1) and preparative HPLC with MeCN–H_2O (1:1) to yield **2** (111 mg) and **6** (91.5 mg). Fraction Ib was submitted to a silica gel column eluting with $CHCl_3$–MeOH (9:1) and preparative HPLC with MeCN–H_2O (11:9) to furnish **3** (81.7 mg) and **4** (11.5 mg). Fraction III was subjected to CC on ODS silica gel eluting with MeOH–H_2O (4:1) to give four additional fractions (IIIa–IIId). Fraction IIIa was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (50:10:1) and ODS silica gel with MeOH–H_2O (7:3) to afford a mixture of **1** and **5**, which was then separated by preparative HPLC using MeCN–H_2O (2:3) to yield **1** (520 mg) and **5** (485 mg).

3.4. Compound *1*

Amorphous solid. $[\alpha]_D^{26}$ −39.0° (MeOH: c 0.50). Positive-ion HRFABMS m/z 603.3891 $[M+Na]^+$ ($C_{33}H_{56}O_8Na$ requires 603.3873). IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 2945 (CH). ^{1}H NMR (C_5D_5N): δ 5.51 (1H, br d, J = 4.9 Hz, H-6), 5.26 (1H, br s, H-1′), 4.47 (1H, br d, J = 3.3 Hz, H-2′), 4.30 (1H, dd, J = 9.3, 3.3 Hz, H-3′), 1.68 (3H, d, J = 6.0 Hz, Me-6′), 1.41 (3H, s, Me-19), 1.22 (3H, d, J = 6.9 Hz, Me-21), 1.04 (3H, s, Me-18), 0.85 (3H × 2, d, J = 6.5 Hz, Me-26 and Me-27). ^{13}C NMR: Table 2.

3.5. Acid hydrolysis of *1*

A solution of **1** (15 mg) in 1 M HCl (dioxane–H_2O,

Table 2
^{13}C NMR spectral data for compounds **1**–**6** in C_5H_5N

C	1	2	3	4	5	6
1	40.1	40.0	40.0	40.0	40.0	40.0
2	32.3	32.2	32.2	32.2	32.3	32.2
3	71.7	71.7	71.7	71.7	71.7	71.7
4	44.2	44.1	44.1	44.2	44.1	44.1
5	142.9	142.9	143.0	142.9	142.9	142.9
6	120.9	120.7	120.8	120.8	120.8	120.7
7	32.9	32.9	32.9	32.9	32.9	32.9
8	31.9	31.7	31.8	31.7	31.8	31.8
9	57.2	57.0	57.1	57.1	57.2	57.0
10	38.9	38.8	38.8	38.8	38.8	38.8
11	68.2	68.1	68.1	68.1	68.2	68.1
12	51.9	51.8	51.8	51.8	51.9	51.8
13	43.0	42.8	42.9	42.9	43.0	42.9
14	54.6	54.4	54.4	54.4	54.5	54.4
15	35.6	35.4	35.4	35.4	35.6	35.7
16	82.4	83.3	83.6	83.3	82.3	83.2
17	57.9	57.7	57.7	57.7	57.8	57.7
18	14.4	14.2	14.3	14.3	14.4	14.3
19	19.3	19.2	19.3	19.3	19.3	19.3
20	36.0	36.1	36.2	36.2	35.1	35.4
21	11.9	12.0	12.0	12.1	11.8	12.0
22	73.2	72.7	72.7	72.7	73.1	72.0
23	34.4	34.5	34.6	34.6	35.3	35.4
24	36.8	36.6	36.6	36.6	123.0	123.5
25	28.7	29.0	29.0	29.0	132.4	132.2
26	22.9	22.8	22.9	22.9	25.9	26.0
27	22.8	22.9	22.9	23.0	18.1	18.1
1′	104.9	101.2	101.2	101.3	104.9	101.2
2′	72.4	71.3	71.5	71.5	72.0	71.5
3′	72.7	72.9	73.8	73.2	72.6	72.9
4′	74.0	71.0	71.3	71.2	74.0	71.0
5′	71.0	70.9	71.0	71.0	70.9	71.0
6′	18.4	18.1	18.2	18.2	18.4	18.1
Ac		170.1	170.0	170.0		170.2
		20.7	20.8	20.7		20.8
		170.4			170.5	
		20.8			20.9	
1″			125.9	123.5		
2″			107.7	132.0		
3″			153.6	114.1		
4″			143.0	163.8		
5″			153.6	114.1		
6″			107.7	132.0		
7″			165.9	165.9		
OMe			60.6	55.4		
			56.0	(×2)		

1:1, 5 ml) was heated at 100°C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Japan) column and chromatographed on silica gel eluting with $CHCl_3$–MeOH (19:1; 1:1) to give an aglycone (9 mg) and L-rhamnose (2 mg). L-Rhamnose was identified by HPLC analysis following their conversion to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives (Kuroda et al., 1997; Oshima, Yamauchi & Kumanotani, 1982). R_t (min): 20.61.

3.6. Compound *1a*

Amorphous solid. $[\alpha]_D^{26}$ −44.0° (MeOH: *c* 0.30). EIMS *m/z* 434 $[M]^+$. IR ν_{max} (KBr) cm^{-1}: 3370 (OH), 2930 (CH). ^1H NMR and ^{13}C NMR spectra: Table 1.

3.7. (*R*),(*S*)-MTPA derivatives of *1a*

Compound **1a** (1.5 mg) was dissolved in dry CH_2Cl_2 (1.5 ml), to which were added (*R*)-MTPA (16.5 mg), 4-(dimethylamino)pyridine (15.9 mg) and 1-ethly-3-(3-dimethylaminopropyl)carbodiimide·HCl (41.2 mg), and the solution was allowed to stand at room temperature for 24 h under an Ar atmosphere. The reaction mixture was diluted with H_2O and extracted with Et_2O. The Et_2O phase was chromatographed on silica gel eluting with hexane–EtOAc (6:1) to give the (*R*)-MTPA ester (**1b**) (0.8 mg) of **1a**. Following this procedure, **1a** (1.5 mg) was converted to the (*S*)-MTPA ester (**1c**) (2 mg) of **1a**. ^1H NMR of **1b** ($CDCl_3$): δ 5.47 (1H, d, *J* = 5.7 Hz, H-6), 5.39 (1H, t-like, *J* = 7.0 Hz, H-22), 4.90 (1H, m, H-3), 4.36 (1H, m, H-16), 4.00 (1H, m, H-11), 3.57 (3H × 2, s, OMe × 2), 2.13 (3H, m, H-20), 1.14 (3H, s, H-19), 0.93 (3H, d, *J* = 6.9 Hz, Me-21), 0.87 (3H, d, *J* = 6.3 Hz, Me-26 or Me-27), 0.86 (3H, s, Me-18), 0.85 (3H, d, *J* = 6.5 Hz, Me-26 or Me-27). ^1H NMR of **1c** ($CDCl_3$): δ 5.49 (1H, d, *J* = 5.6 Hz, H-6), 5.41 (1H, t-like, *J* = 6.8 Hz, H-22), 4.89 (1H, m, H-3), 4.42 (1H, m, H-16), 4.01 (1H, m, H-11), 3.57 (3H, s, OMe), 3.51 (3H, s, OMe), 2.16 (3H, m, H-20), 1.16 (3H, s, Me-19), 0.97 (3H, d, *J* = 7.0 Hz, Me-21), 0.90 (3H, s, Me-18), 0.85 (3H, d, *J* = 6.6Hz, Me-26 or Me-27), 0.83 (3H, d, *J* = 6.6 Hz, Me-26 or Me-27).

3.8. Conformational analysis *1a*

See preceding paper (Kuroda et al., 1999) with the following exception of equilibration time period: 100 ps; and temperature bath set at 300 K.

3.9. Compound *2*

Amorphous solid. $[\alpha]_D^{30}$ −28.0° (MeOH: *c* 0.10). Negative-ion FABMS *m/z* 663 $[M–H]^-$, 621 $[M–Ac]^-$, 579 $[M–Ac × 2]^-$. Positive-ion HRFABMS *m/z* 687.4048 $[M + Na]^+$ ($C_{35}H_{58}O_9Na$ requires 687.4084). IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2930 and 2865 (CH), 1745 and 1725 (C=O), 1465, 1365, 1240, 1220, 1125, 1045, 1015, 985, 955, 900, 805. ^1H NMR (C_5D_5N): δ 5.78 (1H, dd, *J* = 9.9, 3.3 Hz, H-3′), 5.64 (1H, dd, *J* = 3.3, 1.3 Hz, H-2′), 5.50 (1H, br d, *J* = 5.2 Hz, H-6), 5.02 (1H, d, *J* = 1.3 Hz, H-1′), 2.10 and 2.00 (each 3H, s, Ac × 2), 1.70 (3H, d, *J* = 6.2 Hz, Me-6′), 1.41 (3H, s, Me-19), 1.22 (3H, d, *J* = 6.9 Hz, Me-21), 1.08 (3H, d, *J* = 6.5 Hz, Me-26 or Me-27), 1.02 (3H, d,

J = 6.5 Hz, Me-26 or Me-27), 0.99 (3H, s, Me-18). ^{13}C NMR: Table 2.

3.10. Alkaline methanolysis of **2**

Compound **2** (5 mg) was treated with 3% NaOMe in MeOH (2 ml) at room temperature for 1 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo, Japan) column and then chromatographed on silica gel eluting with $CHCl_3$–MeOH (4:1) to yield **1** (3.2 mg).

3.11. Compound **3**

Amorphous solid. $[\alpha]_D^{27}$ +6.0° (MeOH: c 0.10). Negative-ion FABMS m/z 815 $[M-H]^-$. Positive-ion HRFABMS m/z 817.4719 $[M+H]^+$ ($C_{45}H_{69}O_{13}$ requires 817.4738). IR ν_{max} (KBr) cm^{-1}: 3440 (OH), 2930 and 2870 (CH), 1745 and 1720 (C=O), 1585 and 1500 (aromatic ring), 1460, 1415, 1330, 1225, 1170, 1125, 1045, 1000, 955, 860, 810. UV λ_{max} (MeOH) nm (log ε): 266 (4.02). ^{1}H NMR (C_5D_5N): δ 7.58 (2H, s, H-2″ and H-6″), 6.09 (1H, dd, J = 9.7, 3.3 Hz, H-3′), 5.82 (1H, dd, J = 3.3, 1.4 Hz, H-2′), 5.52 (1H, br d, J = 5.2 Hz, H-6), 5.11 (1H, d, J = 1.4 Hz, H-1′), 3.89 (3H, s, OMe), 3.73 (3H × 2, s, OMe × 2), 2.18 (3H, s, Ac), 1.77 (3H, d, J = 6.0 Hz, Me-6′), 1.43 (3H, s, Me-19), 1.28 (3H, d, J = 6.9 Hz, Me-21), 1.03 (3H, s, Me-18), 1.02 (3H, d, J = 6.4 Hz, Me-26 or Me-27), 0.97 (3H, d, J = 6.4 Hz, Me-26 or Me-27). ^{13}C NMR: Table 2.

3.12. Alkaline methanolysis of **3**

Compound **3** (10 mg) was hydrolysed as for **2** above to yield **1** (6.5 mg) and methyl 3,4,5-trimethoxybenzoate (1 mg).

3.13. Compound **4**

Amorphous solid. $[\alpha]_D^{30}$ −12.0° (MeOH: c 0.10). Positive-ion HRFABMS m/z 779.4350 $[M+Na]^+$ ($C_{43}H_{64}O_{11}Na$ requires 779.4346). IR ν_{max} (KBr) cm^{-1}: 3440 (OH), 2950 and 2880 (CH), 1750 and 1725 (C=O), 1590 and 1490 (aromatic ring), 1460, 1415, 1325, 1250, 1230, 1215, 1120, 1050, 950, 860, 800. UV λ_{max} (MeOH) nm (log ε): 260 (3.68). ^{1}H NMR (C_5D_5N): δ 8.21 (2H, d, J = 8.7 Hz, H-2″ and H-6″), 6.96 (2H, d, J = 8.7 Hz, H-3″ and H-5″), 6.09 (1H, dd, J = 9.4, 3.1 Hz, H-3′), 5.81 (1H, br d, J = 3.1 Hz, H-2′), 5.53 (1H, br d, J = 4.5 Hz, H-6), 5.10 (1H, br s, H-1′), 3.66 (3H, s, OMe), 2.15 (3H, s, Ac), 1.78 (3H, d, J = 5.8 Hz, Me-6′), 1.44 (3H, s, Me-19), 1.27 (3H, d, J = 6.6 Hz, Me-21), 1.03 (3H, s, Me-18), 1.02 (3H, d, J = 6.2 Hz, Me-26 or Me-27), 0.98 (3H, d, J = 6.2 Hz, Me-26 or Me-27). ^{13}C NMR: Table 2.

3.14. Alkaline hydrolysis of **4**

Compound **4** (3 mg) was treated with 4% KOH in EtOH (2 ml) at room temperature for 1 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B column and then chromatographed on silica gel eluting with $CHCl_3$–MeOH (9:1; 4:1) to yield **1** (1 mg) and *p*-methoxybenzoic acid (0.5 mg).

3.15. Compound **5**

Amorphous solid. $[\alpha]_D^{28}$ −42.0° (MeOH: c 0.54). Positive-ion HRFABMS m/z 601.3735 $[M+Na]^+$ ($C_{33}H_{54}O_8Na$ requires 601.3716). IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 2975 and 2940 (CH). ^{1}H NMR (C_5D_5N): δ 5.53 (1H, 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 d, J = 2.9 Hz, H-2′), 4.30 (1H, dd, J = 9.8, 2.9 Hz, H-3′), 1.69 (3H, s, Me-27), 1.68 (3H, d, J = 6.0 Hz, Me-6′), 1.67 (3H, s, Me-26), 1.43 (3H, s, Me-19), 1.26 (3H, d, J = 6.8 Hz, Me-21), 1.03 (3H, s, Me-18). ^{13}C NMR: Table 2.

3.16. Compound **6**

Amorphous solid. $[\alpha]_D^{30}$ −24.0° (MeOH: c 0.10). Negative-ion FABMS m/z 661 $[M-H]^-$, 619 $[M-Ac]^-$, 577 $[M-Ac \times 2]^-$. Positive-ion HRFABMS m/z 663.4103 $[M+H]^+$ ($C_{37}H_{59}O_{10}$ 663.4108 requires 685.3928). IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 2970, 2930 and 2865 (CH), 1745 and 1725 (C=O), 1445, 1375, 1240, 1130, 1065, 1050, 1020, 985, 960, 810. ^{1}H NMR (C_5D_5N): δ 5.79 (1H, dd, J = 10.1, 2.8 Hz, H-3′), 5.64 (1H, br d, J = 2.8 Hz, H-2′), 5.50 (1H, br d, J = 5.1 Hz, H-6), 5.04 (1H, br s, H-1′), 2.10 (3H, s, Ac), 1.99 (3H, s, Ac), 1.79 (3H, s, Me-27), 1.74 (3H, s, Me-26), 1.70 (3H, d, J = 5.7 Hz, Me-6′), 1.41 (3H, s, Me-19), 1.25 (3H, d, J = 6.6 Hz, Me-21), 0.98 (3H, s, Me-18). ^{13}C NMR: Table 2.

3.17. Alkaline methanolysis of **6**

Compound **6** (5 mg) was hydrolysed as for **2** above to yield **5** (2.5 mg).

3.18. Cell culture and assay for cytostatic activity

See preceding paper (Kuroda et al., 1999).

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Table 3
The GI_{50}, TGI and LC_{50} values of compound **1** against the NCI 60 cell lines[a]

Panel/cell line	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)
Leukemia			
K-562	0.12	0.43	35
MOLT-4	0.028	0.19	13
RPMI-8226	0.016	0.051	11
SR	0.042	0.25	47
Non-small cell lung cancer			
A 549/ATCC	1.5	–	–
HOP-62	0.32	–	–
NCI-H23	27	–	–
NCI-H522	5.3	–	–
Colon cancer			
COLO 205	1.3	4.6	–
HCC-2998	–[b]	–	–
HCT-116	0.18	–	–
HCT-15	–	–	–
HT29	1.8	–	–
KM12	0.41	2.5	14
SW-620	0.14	4.0	–
CNS cancer			
SF-268	1.2	–	–
SF-295	0.021	0.10	–
SF-539	0.015	0.24	4.6
SNB-19	–	–	–
U251	0.010	–	–
Melanoma			
MALME-3M	2.5	–	–
M14	1.2	5.5	71
SK-MEL-2	7.2	–	–
SK-MEL-28	0.22	2.6	8.5
SK-MEL-5	0.74	34	–
UACC-62	0.50	3.3	–
Ovarian cancer			
OVCAR-3	–	–	–
OVCAR-5	6.2	–	–
OVCAR-8	–	–	–
SK-OV-3	–	–	–
Renal cancer			
786-0	0.11	–	–
A498	0.46	12	–
ACHN	–	–	–
CAKI-1	0.63	41	–
RXF-393	0.025	–	–
SN12C	–	–	–
UO-31	3.1	87	–
Prostate cancer			
PC-3	0.34	–	–
DU-145	–	–	–
Breast cancer			
MCF7	0.022	0.37	10
MCF7/ADR-RES	87	–	–
MDA-MB-231/ATCC	1.0	–	–
MDA-MB-435	0.98	–	–
MDA-N	1.2	–	–
T-47D	–	–	–
Mean value	1.5	20	69

[a] The LC_{50} is the concentration at which only 50% of the cells are viable, the GI_{50} value is the concentration that yields 50% growth and the total growth inhibition (TGI) is the concentration at which no growth was observed.
[b] The value is more than 100 μM.

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