Pergamon

Tetrahedron 58 (2002) 6735–6740

TETRAHEDRON

Lucilianosides A and B, two novel tetranor-lanostane hexaglycosides from the bulbs of *Chionodoxa luciliae*

Minpei Kuroda,[a] Yoshihiro Mimaki,[a,*] Kazutomo Ori,[a] Hiroyuki Koshino,[b] Tomoo Nukada,[b] Hiroshi Sakagami[c] and Yutaka Sashida[a]

[a]*School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan*
[b]*RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*
[c]*Department of Dental Pharmacology, Meikai University School of Dentistry, 1-1 Keyaki-dai, Sakado, Saitama 350-0283, Japan*

Received 17 May 2002; accepted 13 June 2002

Abstract—Two novel hexaglycosides based upon the pentacyclic tetranor-lanostane skeleton with a γ-lactone system, designated as lucilianoside A (**1**) and B (**2**), were isolated from the bulbs of *Chionodoxa luciliae*. Their chemical structures were determined by spectroscopic analysis, including extensive 1D and 2D NMR spectroscopic data, and the results of acid hydrolysis. Lucilianosides A and B exhibited a weak cytotoxicity against HSC-2 cells.

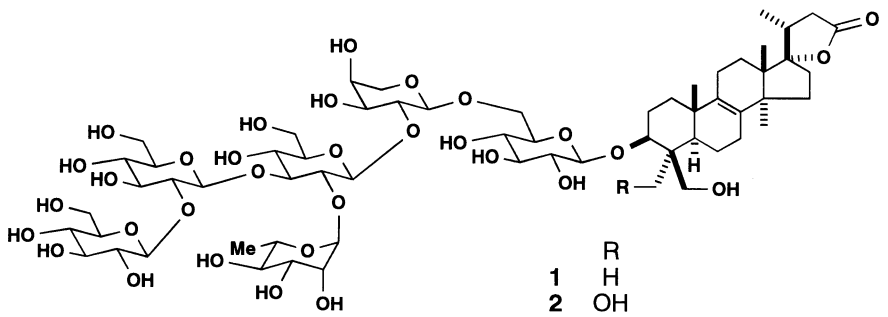
1. Introduction

We have extensively examined the chemical constituents of plants belonging to the subfamily Schilloideae in the Liliaceae and isolated a variety of steroidal and triterpenoid glycosides, some of which were unique in structure and showed significant biological activities. For example, an acylated cholestane glycoside from *Ornithogalum saundersiae*, is a possible candidate for a new anticancer agent.[1] Peruvianosides A and B, the pentacyclic lanostane triglycosides isolated from *Scilla peruviana*, have a rearranged side-chain moiety and are interesting from the phytochemical view point.[2] Up to now, the lanostane glycosides with a spiro-lactone ring system and nor-lanostane glycosides were isolated from several *Scilla*, *Eucomis*, and *Chionodoxa* species,[3] and showed tumor-promoter inhibitory activity.[4] The present investigation of the bulbs of *Chionodoxa luciliae* Boiss. is part of a series of studies on the chemical constituents of plants of the Schilloideae. As a result, two novel hexaglycosides having the pentacyclic tetranor-lanostane skeleton with a γ-lactone system, named lucilianoside A (**1**) and B (**2**), were isolated, and their chemical structures were determined on the basis of spectroscopic analysis, including extensive 1D and 2D NMR spectroscopic data, and the results of acid hydrolysis. Lucilianosides A and B were evaluated for their cytotoxic activity against human oral squamous cell carcinoma (HSC-2) cells.

2. Results and discussion

The MeOH extract of the bulbs of *C. luciliae* was repeatedly subjected to Si gel and octadecylsilanized (ODS) Si gel column chromatography, as well as to preparative HPLC, giving lucilianosides A (**1**) and B (**2**).

Lucilianoside A (**1**) was obtained as an amorphous solid and its molecular formula was deduced as $C_{61}H_{98}O_{32}$ from its



Keywords: *Chionodoxa luciliae*; tetranor-lanostane; hexaglycosides; cytotoxicity.
* Corresponding author. Tel.: +81-426-76-4577; fax: +81-426-76-4579; e-mail: mimakiy@ps.toyaku.ac.jp

0040–4020/02/$ - see front matter
PII: S0040-4020(02)00672-5

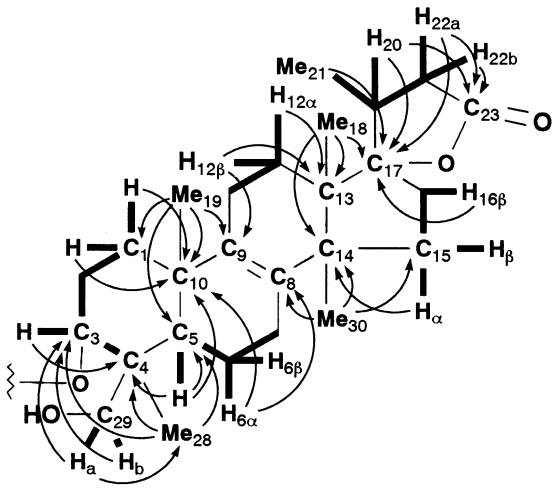


Figure 1. HMBC correlations of aglycon moiety of **1**.

positive and negative-ion FABMS, which showed an $[M+Na]^+$ ion at *m/z* 1365 and $[M-H]^-$ ion at *m/z* 1341, ^{13}C NMR spectral and elemental analysis data. The ^1H NMR spectrum, measured in a mixture of C_5D_5N and CD_3OD (11:1), showed signals for five triterpene methyl groups at δ 1.54 (s), 1.20 (s), 1.04 (d, J=6.8 Hz), 0.92 (s), and 0.88 (s), together with signals for six anomeric protons at δ 6.38 (br s), 5.32 (d, J=7.8 Hz), 5.26 (d, J=3.4 Hz), 5.16 (d, J=7.8 Hz), 5.07 (d, J=7.8 Hz), and 4.94 (d, J=7.8 Hz). In the ^{13}C NMR spectrum, six anomeric carbons were observed at δ 106.6, 106.2, 102.5, 101.6, 101.5, and 101.4. The methyl carbon signal at δ 18.6 and proton signal at δ 1.80 (d, J=7.8 Hz) were suggestive of **1** having one deoxy sugar. Acid hydrolysis of **1** with 1 M HCl in dioxane-H_2O (1:1) gave D-glucose, L-arabinose, and L-rhamnose in a ratio of 4:1:1 as the carbohydrate moieties, while the labile aglycon was decomposed under acid conditions. The ^{13}C NMR data of **1** showed a total of 61 signals, 35 of which were assigned to four glucosyl, one arabinosyl, and one rhamnosyl units. This led to a $C_{26}H_{40}O_4$ composition for the aglycon moiety, possessing seven degrees of unsaturation. The presence of a pair of double bond (δ_C 135.2 and 134.9) and a carbonyl group (δ_C 177.2) accounted for two degrees. Consequently, the aglycon was predicated to have a C_{26} terpenoid skeleton with a five-ring system. A combination of the $^1H-^1H$ COSY and HMQC experiments allowed the full assignments of the proton and carbon signals of the aglycon part and showed the following structural fragments $-C_{(1)}H_2-C_{(2)}H_2-C_{(3)}H(-O-)$ for ring A, $-C_{(5)}H-C_{(6)}H_2-C_{(7)}H_2-$ for ring B, $-C_{(11)}H_2-C_{(12)}H_2-$ for ring C, $-C_{(15)}H_2-C_{(16)}H_2-$ for ring D, and $Me_{(21)}-C_{(20)}H-C_{(22)}H_2-$ for ring E, starting from well solved signals at δ 3.57 (H-3), 1.24 (H-5), 2.22 (H-12α), and 1.32 (H-15α). The HMBC correlations from H_2-1 (δ 1.67 and 1.14) to C-10 (δ 37.0), H-3 to C-4 (δ 45.6), H-5 to C-4 and C-10, and from Me-19 to C-1 (δ 35.8), C-5 (δ 51.9), and C-10, established the connections of ring A and ring B via the C-4 and C-10 quaternary carbons, and of C-19 methyl and C-10 (Fig. 1). The signals at δ 4.39 and 3.63 (J=10.3 Hz), which were associated with the carbon signal at δ 63.3, were consistent with the presence of a hydroxymethyl group. The long-range correlations from the δ 4.39 resonance to C-28 (δ 23.3) and from Me-28 (δ 1.54) to C-4 revealed that a methyl group and a hydroxymethyl group were geminally located at C-4. The connectivity of ring C and ring D, and the respective location of a methyl group at C-13 and C-14 were shown on the basis of the HMBC cross-peaks from H_2-12 (δ 2.22 and 1.46) to C-13 (δ 49.1), H-15α (δ 1.32) to C-14 (δ 51.0), Me-18 (δ 0.88) to C-13, and from Me-30 (δ 1.20) to C-14 and C-15 (δ 31.8). The HMBC correlations between H-6α (δ 1.81) and C-8 (δ 134.9), and between H-12β (δ 1.46) and C-9 (δ 135.2) confirmed the presence of the C-8/C-9 double bond and the connection of ring B and ring C. The presence of a γ-lactone ring was suggested by the IR (1745 cm^{-1}) and ^{13}C NMR (δ 177.2) spectra. In the HMBC spectrum, the cross-peaks from H-20 (δ 2.36), Me-21 (δ 1.04), and H-22a (δ 2.07) to the downfield-shifted quaternary carbon at δ 98.3 assignable to C-17, and those from H-20, H-22a, and H-22b (δ 2.81) to C-23 (δ 177.2) gave confirmative evidence for the formation of a γ-lactone ring between C-17 and C-23. The connection of ring D and the γ-lactone moiety via C-17 was confirmed by an HMBC correlation from H-16β (δ 2.08) to C-17. Thus, the plane structure of the aglycon moiety of **1** was shown to be 3,29-dihydroxy-24,25,26,27-tetranor-lanost-8-en-17,23-olide. Analysis of the phase-sensitive NOESY spectrum made the relative stereochemistry assignable (Fig. 2). The NOE correlations from Me-19 to H-2β (δ 1.98), H-6β (δ 1.48), H-11β (δ 1.95), H_2-29, and from H-5 to H-1α, H-3, and Me-28 indicated that **1** had the A/B *trans* ring junction, and the Me-28α and CH_2-29β configurations. Furthermore, NOEs from Me-18 to H-11β and H-15β (δ 1.67), and from Me-30 to H-7α (δ 2.03) and H-15α confirmed the C/D *trans* ring junction. NOEs between H-16β and H-20, and between H-12β and Me-21 were consistent with the 17*S* and 20*R* configurations. The orientation of the oxygen atom at C-3, to which the hexaglycoside group were linked, was

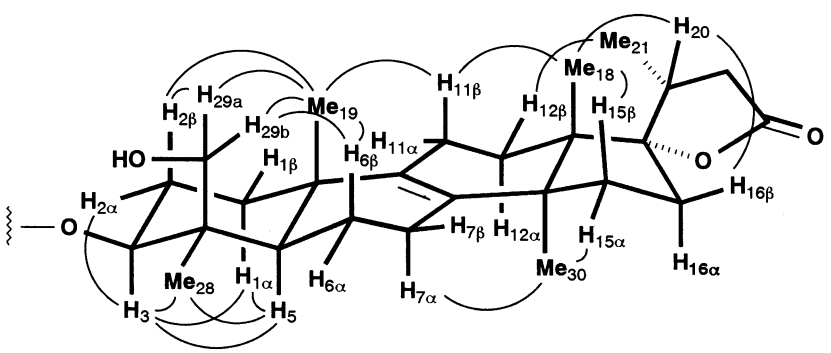


Figure 2. NOE correlations of aglycon moiety of **1**.

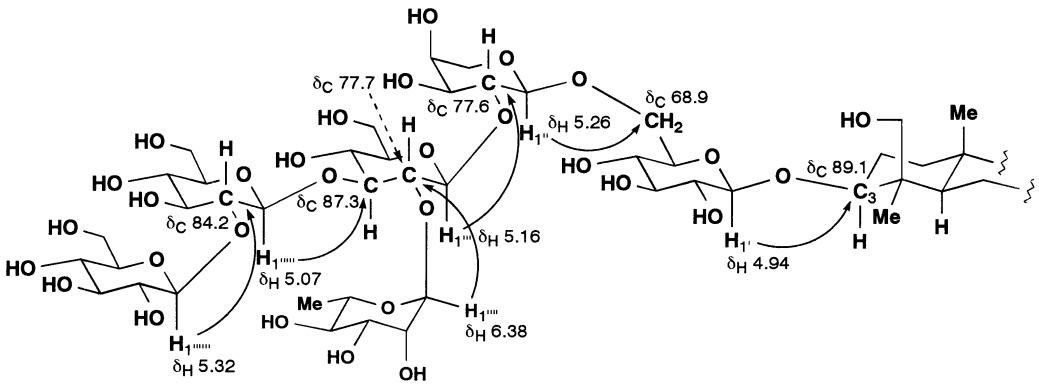


Figure 3. HMBC correlations of the hexaglycoside moiety of **1**.

determined to be β from the H-3 proton coupling constants (dd, J=11.7, 4.4 Hz).

The sequence of the hexaglycoside moiety was solved by the concerted use of 1D TOCSY and 2D NMR experiments. Because of the selectivity of the multistep coherence transfer, 1D TOCSY method allowed a sub-spectrum of a single monosaccharide unit to be extracted from the complicated overlapped region. The isolated anomeric proton signals and methyl doublet signal of the rhamnosyl moiety, which resonated in an resolved region of the spectrum, were used for the starting points of the 1D TOCSY experiments. As a result, the sub-spectrum of each sugar residue was obtained with high digital resolution. Subsequent analysis of the $^{1}H-^{1}H$ COSY spectrum resulted in the sequential assignments of all of the proton resonances for the individual monosaccharides. The HMQC spectrum correlated the proton resonances with those of the corresponding one-bond coupled carbons, leading to the unambiguous assignments of the carbon shifts. Comparison of the carbon chemical shift thus assigned with those of the reference methyl glycosides,[5] taking into account the known effects of *O*-glycosylation, indicated that **1** contained a terminal β-D-glucopyranosyl unit, a terminal α-L-rhamnopyranosyl unit, a 2-substituted β-D-glucopyranosyl unit, a 6-substituted β-D-glucopyranosyl unit, a 2,3-disubstituted β-D-glucopyranosyl unit, and a 2-substituted α-L-arabinopyranosyl unit in the molecule. Finally, the $^{3}J_{C,H}$ correlation from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide revealed the exact sugar sequence. In the HMBC spectrum, the anomeric proton signals at δ 6.38 (rhamnosyl), 5.32 (glucosyl), 5.26 (2-substituted arabinosyl), 5.16 (2,3-disubstituted glucosyl), 5.07 (2-substituted glucosyl), and 4.94 (6-substituted glucosyl) showed correlations with the carbon signals at δ 77.7 (C-2 of 2,3-disubstituted glucosyl), 84.2 (C-2 of 2-substituted glucosyl), 68.9 (C-6 of 6-disubstituted glucosyl), 77.6 (C-2 of 2-substituted arabinosyl), 87.3 (C-3 of 2,3-disubstituted glucosyl), and 89.1 (C-3 of aglycon), respectively (Fig. 3). Accordingly, the structure of **1** was elucidated as 29-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-24,25,26,27-tetranorlanost-8-en-17α,23-olide.

Lucilianoside B (**2**) was shown to have the molecular formula $C_{61}H_{98}O_{33}$ on the basis of the negative-ion FABMS (m/z 1357 $[M-H]^{-}$), ^{13}C NMR, and elemental analysis. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** showed their considerable structural similarity. However, the molecular formula of **2** was higher by one oxygen atom than that of **1**. In the ^{13}C NMR spectrum, the signal due to the C-28 methyl, which was observed at δ 23.3 in **1**, was displaced by signal due to a hydroxymethyl carbon at δ 61.1. All other signals were almost superimposable between **1** and **2**. Thus, **2** was proved to be the C-28 hydroxy derivative of **1**. Thus, the structure of **2** was determined to be as 28,29-dihydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-24,25,26,27-tetranor-lanost-8-en-17α,23-olide.

The small $^{3}J_{H\text{-}1,H\text{-}2}$ coupling constant (3.2–3.4 Hz) for the anomeric proton of the arabinopyranosyl moiety of **1** and **2** suggested that it has a $^{1}C_{4}$ conformation to reduce the steric hindrance caused by the C-2 substituted group.[6] However, selective 1D NOESY data obtained by irradiating the anomeric proton signal yielded the sub-spectrum of the arabinosyl residue, which showed NOEs from H-1 to H-2, H-3, and H-5 (Fig. 4). This indicated that the arabinosyl group are present as the $^{1}C_{4}$ and $^{4}C_{1}$ forms in equilibrium with rapid conformational exchange.

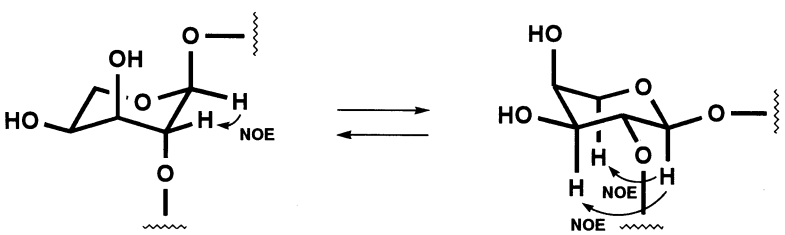


Figure 4. NOE correlations of the arabinosyl moiety observed in the 1D NOESY spectrum.

Although the plants belonging to the subfamily Schilloideae are known to produce a variety of triterpene glycosides based upon the lanostane skeleton,[2–4] lucilianosides A (**1**) and B (**2**) are believed to be the first glycosides of the tetranor-lanostanes with a γ-lactone ring system.

Lucilianosides A and B showed a weak cytotoxic activity against HSC-2 cells with LD_{50} values of 254 and 238 μg/mL, respectively.

3. Experimental

3.1. General

Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 (Tokyo, Japan) spectrophotometer, and MS on a Finnigan MAT TSQ-700 (San Jose, CA, USA), using a dithiothreitol-dithioerythritol (3:1) matrix. Elemental analysis was carried out using an Elementar Vario EL (Hanau, Germany) elemental analyzer. NMR spectra were recorded on a Bruker AM-500 (500 MHz for ^{1}H NMR, Karlsruhe, Germany) or a JEOL JNM-α 600 (600 MHz for ^{1}H NMR, Tokyo, Japan). Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. Si gel (Fuji-Silysia Chemical, Aichi, Japan) and ODS Si gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a HPLC system composed of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh CCP PX-8010 controller, a Tosoh UV-8000 or an RI-8010 detector, and Rheodyne injection port with a 2 mL sample loop for preparative HPLC and a 20 μL sample loop for analytical HPLC. A Capcell Pak C_{18} UG80 column (10 mm i.d.×250 mm, ODS, 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC, and a TSK-gel ODS-Prep column (4.6 mm i.d.×250 mm, ODS, 5 μm, Tosoh) employed for analytical HPLC. The following reagents were obtained from the indicated companies: FBS (JRH Biosciences, Lenexa, KS, USA); Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY, USA); MTT, penicillin, and streptomycin (Sigma, St Louis, MO, USA). All other chemicals used were of biochemical reagent grade.

3.2. Plant material, extraction, and isolation

The bulbs of *C. luciliae* were purchased from a nursery in Heiwaen, Nara Prefecture, Japan. The bulbs were cultivated and the flowered plant was identified by one of the authors (Y. S.). A voucher of the plant is on file in our laboratory (voucher no. CL-94-003, Laboratory of Medicinal Plant Science). The plant material (fresh weight, 3.3 kg) was extracted with hot MeOH. Column chromatography of the MeOH extract on Si gel and elution with a gradient mixture of $CHCl_3$–MeOH system (9:1; 6:1; 4:1; 2:1), and finally with MeOH alone, gave four fractions (I–IV). Fr. III was chromatographed on ODS Si gel eluting with MeOH–H_2O (4:1) into two fractions (Fr. IIIa, IIIb). Fr. IIIa was further separated by a Si gel column eluting with $CHCl_3$–MeOH–H_2O (6:4:1) and preparative HPLC using MeOH–H_2O (3:2) to furnish **1** (43.4 mg). Fr. IIIb was chromatographed on Si gel eluting with $CHCl_3$–MeOH–H_2O (7:4:1) and ODS Si gel with MeCN–H_2O (9:11; 2:3) to give **2** (30.2 mg).

3.2.1. Lucilianoside A (1). An amorphous solid; $[\alpha]_D^{27}=-28.0°$ (*c* 0.10, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2930 and 2880 (CH), 1745 (C=O, γ-lactone), 1450, 1410, 1370, 1300, 1150, 1050, 910; ^{1}H and ^{13}C NMR, see Table 1; FABMS (negative mode) *m/z* 1341 $[M-H]^-$, 1179 $[M-H\text{-glucosyl}]^-$, 1017 $[M-H\text{-glucosyl}\times2]^-$, 871 $[M-H\text{-glucosyl}\times2-\text{rhamnosyl}]^-$; FABMS (positive mode) *m/z* 1365 $[M+Na]^+$; anal. C 50.77%, H 7.82% (calcd for $C_{61}H_{98}O_{32}\cdot6H_2O$, C 50.74%, H 7.62%).

3.3. Acid hydrolysis of 1 and identification of monosaccharides

A solution of **1** (5.0 mg) in 1 M HCl (dioxane-H_2O, 1:1, 5 mL) was heated at 95°C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on Si gel eluting with $CHCl_3$-MeOH (9:1; 1:1) to give an aglycon fraction (1.2 mg) and a sugar fraction (3.0 mg). TLC analysis of the aglycon fraction showed that it contained several unidentified artifactual sapogenols. The sugar fraction was dissolved in H_2O (1 mL), to which (−)-α-methylbenzylamine (5 mg) and $Na[BH_3CN]$ (8 mg) in EtOH (1 mL) were added. After being set aside at 40°C for 4 h followed by addition of AcOH (0.2 mL) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 mL) in pyridine (0.3 mL) at room temperature for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) with H_2O–MeCN (4:1; 1:1; 1:9, each 5 mL) mixtures as solvents. The H_2O–MeCN (1:9) eluate was further passed through a Toyopak IC-SP M cartridge (Tosoh) with EtOH (10 mL) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides,[7] which was then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2:3); flow rate, 0.8 mL/min; detection, UV 230 nm. The derivatives of D-glucose, L-arabinose, and L-rhamnose were detected; t_R (min): 13.41 (derivative of L-arabinose); 17.84 (derivative of D-glucose); 20.06 (derivative of L-rhamnose).

3.3.1. Lucilianoside B (2). An amorphous solid; $[\alpha]_D^{27}=-36.0°$ (*c* 0.10, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2930 and 2890 (CH), 1745 (C=O, γ-lactone), 1450, 1420, 1370, 1310, 1150, 1070, 1040, 915, 780; ^{1}H NMR (C_5D_5N): δ 6.43 (1H, br s, H-1$''''$), 5.35 (1H, d, *J*=7.7 Hz, H-1$''''''$), 5.27 (1H, d, *J*=3.2 Hz, H-1$''$), 5.13 (1H, d, *J*=7.5 Hz, H-1$'''$), 5.07 (1H, d, *J*=7.6 Hz, H-1$'''''$), 4.98 (1H, d, *J*=7.8 Hz, H-1$'$), 1.83 (3H, d, *J*=6.1 Hz, Me-6$''''$), 1.14 (3H, s, Me-30), 1.03 (3H, s, Me-19), 1.01 (3H, d, *J*=6.9 Hz, Me-21), 0.88 (3H, s, Me-18); ^{13}C NMR (C_5D_5N): δ 35.6 (C-1), 27.2 (C-2), 82.1 (C-3), 48.2 (C-4), 43.4 (C-5), 18.6 (C-6), 26.5 (C-7), 134.8 (C-8), 135.3 (C-9), 36.7

Table 1. ^{1}H and ^{13}C NMR spectral data for compound **1** in C_5D_5N–CD_3OD (11:1)

	^{1}H	*J* (Hz)	^{13}C		^{1}H	*J* (Hz)	^{13}C
1 ax	1.14 ddd	13.2, 11.2, 3.4	35.8	Ara (2-substituted)			
1 eq	1.67 m			1″	5.26 d	3.4	101.5
2 ax	1.98 m		27.6	2″	4.67 dd	5.9, 3.4	77.6
2 eq	2.27 m			3″	4.64 dd	5.9, 3.2	71.7
3	3.57 dd	11.7, 4.4	89.1	4″	4.50 ddd	6.8, 3.4, 3.2	67.1
4			45.6	5″	4.35 dd	11.7, 6.8	63.1
5	1.24 br d	14.2	51.9		3.92 dd	11.7, 3.4	
6 α	1.81 m		18.9	Glc (2,3-disubstituted)			
6 β	1.48 m			1‴	5.16 d	7.8	102.5
7 α	2.03 m		27.0	2‴	4.25 dd	9.0, 7.8	77.7
7 β	1.90 m			3‴	4.13 t-like	9.0	87.3
8			134.9	4‴	4.14 t-like	9.0	68.6
9			135.2	5‴	3.63 ddd	8.8, 4.9, 2.9	77.8
10			37.0	6‴	4.23 dd	11.0, 2.9	62.0
11 α	2.13 m		21.0		4.14 dd	11.0, 4.9	
11 β	1.95 m			Rha (terminal)			
12 α	2.22 m		24.9	1⁗	6.38 br s		101.6
12 β	1.46 m			2⁗	4.69 dd	2.5, 1.0	72.3
13			49.1	3⁗	4.53 dd	9.3, 2.5	72.5
14			51.0	4⁗	4.24 dd	9.3, 9.3	74.0
15 α	1.32 m		31.8	5⁗	4.89 dd	9.3, 5.9	70.0
15 β	1.67 m			6⁗	1.80 d	5.9	18.6
16 α	1.98 m		39.3	Glc (2-substituted)			
16 β	2.08 m			1⁗′	5.07 d	7.8	101.4
17			98.3	2⁗′	4.05 dd	9.0, 7.8	84.2
18	0.88 s		18.1	3⁗′	4.20 dd	9.3, 9.0	78.3
19	0.92 s		19.6	4⁗′	4.03 dd	9.3, 9.3	70.9
20	2.36 dq	6.4, 6.8	42.0	5⁗′	3.87 ddd	9.3, 6.4, 3.2	78.4
21	1.04 d	6.8	17.8	6⁗′	4.43 dd	11.2, 6.4	62.1
22 a	2.07 d	16.6	39.3		4.20 dd		
22 b	2.81 dd	16.6, 6.4		Glc (terminal)			
23			177.2	1⁗″	5.32 d	7.8	106.6
28	1.54 s		23.3	2⁗″	4.24 dd	9.0, 7.8	76.1
29 a	4.39 d	10.3	63.3	3⁗″	4.13 dd	9.3, 9.0	78.1
29 b	3.63 d	10.3		4⁗″	4.24 dd	9.3, 9.3	70.6
30	1.20 s		25.9	5⁗″	3.83 ddd	9.3, 3.4, 2.4	78.9
Glc (6-substituted)				6⁗″	4.37 dd	11.7, 2.4	62.2
1′	4.94 d	7.8	106.2		4.28 dd	11.7, 3.4	
2′	3.94 dd	9.0, 7.8	75.5				
3′	4.15 t-like	9.0	78.3				
4′	4.15 t-like	9.0	72.5				
5′	4.01 ddd	9.0, 4.4, 3.4	75.8				
6′	4.53 dd	12.6, 3.4	68.9				
	4.27 dd	12.6, 4.4					

(C-10), 20.9 (C-11), 24.7 (C-12), 49.0 (C-13), 50.8 (C-14), 31.6 (C-15), 29.8 (C-16), 97.9 (C-17), 18.0 (C-18), 19.5 (C-19), 41.7 (C-20), 17.6 (C-21), 39.1 (C-22), 176.7 (C-23), 61.1 (C-28), 62.7 (C-29), 25.6 (C-30), 105.5 (C-1′), 75.4 (C-2′), 78.1 (C-3′), 72.5 (C-4′), 75.4 (C-5′), 68.7 (C-6′), 101.1 (C-1″), 77.4 (C-2″), 71.4 (C-3″), 66.7 (C-4″), 62.6 (C-5″), 102.4 (C-1‴), 77.5 (C-2‴), 87.4 (C-3‴), 68.3 (C-4‴), 77.6 (C-5‴), 61.8 (C-6‴), 101.4 (C-1⁗), 72.2 (C-2⁗), 72.4 (C-3⁗), 74.0 (C-4⁗), 69.9 (C-5⁗), 18.5 (C-6⁗), 101.1 (C-1⁗′), 84.5 (C-2⁗′), 78.2 (C-3⁗′), 70.8 (C-4⁗′), 78.2 (C-5⁗′), 62.0 (C-6⁗′), 106.7 (C-1⁗″), 76.0 (C-2⁗″), 77.8 (C-3⁗″), 70.5 (C-4⁗″), 78.8 (C-5⁗″), 62.0 (C-6⁗″); FABMS (negative mode) *m/z* 1357 $[M-H]^-$; FABMS (positive mode) *m/z* 1381 $[M+Na]^+$; anal. C 50.0%, H 7.82% (calcd for $C_{61}H_{98}O_{33}\cdot 6H_2O$, C 49.93%, H 7.56%).

3.4. Acid hydrolysis of 2

Compound **2** (5.0 mg) was subjected to acid hydrolysis by the same procedures as described for **1** to give a sugar fraction (2.5 mg). The monosaccharide constituents in the sugar fraction were converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives, which were then analyzed by HPLC. The derivatives of L-arabinose, D-glucose, and L-rhamnose were detected.

3.5. HSC-2 cell culture assay

HSC-2 cells were maintained as monolayer cultures at 37°C in DMEM supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified 5% CO_2 atmosphere. Cells were trypsinized and inoculated at 6×10^3 per each 96-microwell plate (Falcon, flat bottom, treated polystyrene, Becton Dickinson, San Jose, CA, USA), and incubated for 24 h. After washing once with phosphate-buffered saline (PBS, 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.4), they were treated with 24 h without or with test compounds. They were washed once with PBS and incubated for 4 h with 0.2 mg/mL MTT in DMEM supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 mL DMSO and

the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan® (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10.[8]

Acknowledgments

We are grateful to Dr Y. Shida and Mr H. Fukaya, Tokyo University of Pharmacy and Life Science, for the measurements of the mass spectra and elemental analysis.

References

1. Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633–636.
2. (a) Mimaki, Y.; Ori, K.; Sashida, Y.; Nikaido, T.; Song, L.-G.; Ohmoto, T. *Bull. Chem. Soc. Jpn* **1993**, *66*, 1182–1186. (b) Mimaki, Y.; Ori, K.; Sashida, Y.; Nikaido, T.; Song, L.-G.; Ohmoto, T. *Chem. Lett.* **1992**, 1999–2000.
3. (a) Mimaki, Y.; Ori, K.; Kubo, S.; Sashida, Y.; Niakido, T.; Song, L.-G.; Ohmoto, T. *Chem. Lett.* **1992**, 1863–1866. (b) Mimaki, Y.; Kubo, S.; Kinoshita, Y.; Sashida, Y.; Song, L.-G.; Niakido, T.; Ohmoto, T. *Phytochemistry* **1993**, *34*, 791–797.
4. Mimaki, Y.; Nishino, H.; Ori, K.; Kuroda, M.; Matsui, T.; Sashida, Y. *Chem. Pharm. Bull.* **1994**, *42*, 327–332.
5. (a) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479–2496. (b) Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307–3330.
6. (a) Ishii, H.; Kitagawa, I.; Matsushita, K.; Shirakawa, K.; Tori, K.; Tozyo, T.; Yoshikawa, M.; Yoshimura, Y. *Tetrahedron Lett.* **1981**, 1529–1535. (b) Tanaka, O. *Yakugaku Zasshi* **1985**, *105*, 323–351.
7. (a) Oshima, R.; Kumanotani, J. *Chem. Lett.* **1981**, 943–946. (b) Oshima, R.; Yamauchi, Y.; Kumanotani, J. *Carbohydr. Res.* **1982**, *107*, 169–176.
8. (a) Furuya, S.; Takayama, F.; Mimaki, Y.; Sashida, Y.; Satoh, K.; Sakagami, H. *Anticancer Res.* **2000**, *20*, 4189–4194. (b) Furuya, S.; Takayama, F.; Mimaki, Y.; Sashida, Y.; Satoh, K.; Sakagami, H. *Anticancer Res.* **2001**, *21*, 959–964.