Norlanostane and Lanostane Glycosides from the Bulbs of *Chionodoxa luciliae* and Their Cytotoxic Activity

Kazutomo Ori,[a] Minpei Kuroda,[a] Yoshihiro Mimaki,*,[a] Hiroshi Sakagami,[b] and Yutaka Sashida[a]

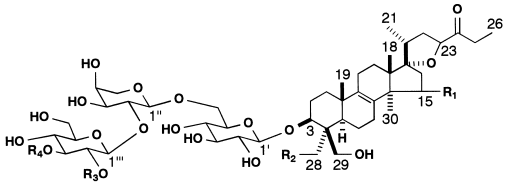
[a] *Laboratory of Medicinal Plant Science, School of Pharmacy, Tokyo University of Pharmacy and Life Science; 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan: and* [b] *Department of Dental Pharmacology, Meikai University School of Dentistry; 1–1 Keyaki-dai, Sakado, Saitama 350–0283, Japan.* Received September 2, 2002; accepted October 16, 2002

Ten lanostane glycosides (1—10), including two new norlanostane glycosides (2 and 7) and a new lanostane glycoside with a spirolactone ring system (9), were isolated from the fresh bulbs of *Chionodoxa luciliae* (Liliaceae). The structures of the new compounds were determined on the basis of extensive spectroscopic analysis and the results of hydrolytic cleavage to be (23*S*)-3β-[(*O*-β-D-apiofuranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-17α,23-epoxy-28,29-dihydroxy-27-norlanost-8-en-24-one (2), (23*S*)-17α,23-epoxy-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)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-27-norlanost-8-ene-15,24-dione (7), and (23*S*,25*R*)-17α,23-epoxy-29-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]lanost-8-en-23,26-olide (9), respectively. The cytotoxic activity of the isolated compounds against HSC-2 human oral squamous cell carcinoma cells are also reported.

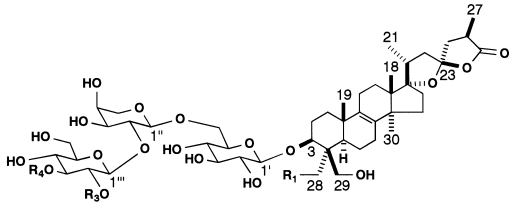
Key words *Chionodoxa luciliae*; Liliaceae; norlanostane glycoside; lanostane glycoside; cytotoxic activity; HSC-2 cell

A variety of oligoglycosides of triterpenes with the lanostane skeleton have been isolated from the *Scilla*, *Eucomis*, and *Chionodoxa* species in the family Liliaceae, and some are unique in structure. Peruvianosides A and B from *Scilla peruviana* are pentacyclic lanostane triglycosides with a rearranged lanostane side chain moiety.[1,2] Scillasaponin A from *Eucomis bicolor*, B from *S. peruviana*, and C from *Chionodoxa gigantea* are new lanosterol oligoglycosides with modification of the side chain to form a spirolactone ring system.[3] Most recently, we have isolated and characterized two novel hexaglycosides having a pentacyclic tetranorlanostane skeleton with a γ-lactone ring system, called lucilianosides A and B,[4] from *Chionodoxa luciliae*. Further chemical investigation of the bulbs of *C. luciliae* has led to the isolation of 10 lanostane glycosides (**1**—**10**), including two new norlanostane glycosides (**2** and **7**) and a new lanostane glycoside with a spirolactone ring system (**9**). In this paper, we report the structural assignment of the new compounds on the basis of extensive spectroscopic analysis and the results of hydrolytic cleavage. The cytotoxicity of the isolated compounds against HSC-2 human oral squamous cell carcinoma cells is also described.

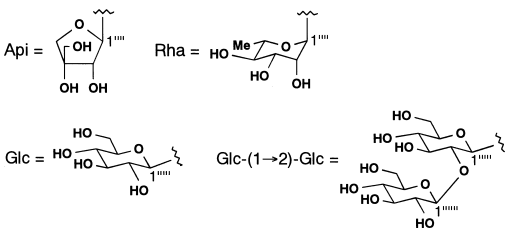
Fresh bulbs of *C. luciliae* were extracted with hot MeOH. The concentrated MeOH extract was repeatedly subjected to silica gel and octadecylsilanized (ODS) silica gel column chromatography, as well as to preparative HPLC, yielding compounds **1**—**10**. Compounds **1**, **3**—**6**, **8**, and **10** were identified as (23*S*)-3β-[(*O*-β-D-apiofuranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-17α,23-epoxy-28,29-dihydroxy-27-norlanost-8-en-24-one (**1**),[5] (23*S*)-17α,23-epoxy-28,29-dihydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-27-norlanost-8-en-24-one (**3**),[6] (23*S*)-17α,23-epoxy-29-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-27-norlanost-8-en-24-one (**4**),[7] (23*S*)-17α,23-epoxy-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)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-27-norlanost-8-en-24-one (**5**),[8] (23*R*)-17α,23-epoxy-29-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyra-



		R_1	R_2	R_3	R_4
1	(23*S*)	H,H	HO	Api	H
2	(23*S*)	H,H	HO	Api	Glc
3	(23*S*)	H,H	HO	Rha	H
4	(23*S*)	H,H	H	Rha	Glc
5	(23*S*)	H,H	H	Rha	Glc-(1→2)-Glc
6	(23*R*)	H,H	H	Rha	Glc-(1→2)-Glc
7	(23*S*)	=O	H	Rha	Glc-(1→2)-Glc



	R_1	R_3	R_4
8	HO	Api	H
9	H	Rha	Glc
10	H	Rha	Glc-(1→2)-Glc



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

nosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]-27-norlanost-8-en-24-one (**6**),[8] (23*S*,25*R*)-3*β*-[(*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]-17*α*,23-epoxy-28,29-dihydroxylanost-8-en-23,26-olide (**8**),[5] and (23*S*,25*R*)-17*α*,23-epoxy-29-hydroxy-3*β*-[(*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]lanost-8-en-23,26-olide (**10**),[3,8] respectively.

Compound **2** was obtained as an amorphous solid with a molecular formula of $C_{57}H_{92}O_{28}$, deduced from elemental analysis combined with the data of the negative-ion FAB-MS, which showed an $[M-H]^-$ ion at *m*/*z* 1223, ^{13}C-NMR spectrum with a total of 57 carbon signals, and distortionless enhancement by polarization transfer (DEPT) spectra. The IR spectrum showed absorption bands of hydroxyl groups at 3400 cm^{-1} and a carbonyl group at 1715 cm^{-1}. The ^{1}H-NMR spectrum displayed a three-proton doublet signal at δ 1.02 (J=6.4 Hz) and three three-proton singlet signals at δ 1.47, 1.08, and 0.93, as well as a three-proton triplet signal at δ 1.04 (J=7.3 Hz), which is characteristic of the 27-norlanostane triterpene derivatives. These ^{1}H-NMR data and comparison of the ^{13}C-NMR spectrum with that of **1** indicated that the aglycon of **2** was identical to that of **1**. Acid hydrolysis of **2** with 0.2 M HCl in dioxane–H_2O (1 : 1) gave D-apiose, L-arabinose, and D-glucose as the carbohydrate moieties, while the labile aglycon was decomposed under acid conditions. The monosaccharides, including their absolute configurations, were identified by HPLC analysis following their conversion to the 1-[(*S*)-*N*-acetyl-*α*-methylbenzylamino]-1-deoxyalditol acetate derivatives.[9] The molecular formula of **2** was higher by $C_6H_{10}O_5$ than that of **1**, and the ^{1}H-NMR spectrum of **2** showed signals for five anomeric protons at δ 6.24 (d, J=4.0 Hz), 5.15 (d, J=7.9 Hz), 5.12 (d, J=7.6 Hz), 5.06 (br s), and 5.05 (d, J=7.5 Hz), suggesting the presence of one more hexose unit in **2**. On comparison of the entire ^{13}C-NMR spectrum of **2** with that of **1**, a set of six additional signals corresponding to a terminal *β*-D-glucopyranosyl moiety appeared at δ 104.6 (CH), 74.9 (CH), 78.5 (CH), 72.5 (CH), 78.3 (CH), and 62.4 (CH_2),[10] and the signals due to C-3 of the glucosyl moiety attached at C-2 of the arabinosyl residue and its neighboring carbons varied, while all other signals remained almost unaffected (Table 1). The exact sugar sequences of **2** were determined by the following ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) correlations. The anomeric proton signal of the terminal glucosyl group at δ 5.05 showed a long-range correlation with C-3 of the branched glucosyl moiety at δ 88.2, of which the anomeric proton at δ 5.15 in turn exhibited a correlation with C-2 of the arabinosyl unit at δ 77.6. The apiofuranosyl moiety was considered to be a terminal unit because of the absence of any glycosylation shift for their carbon signals, and its anomeric proton showed an HMBC correlation with C-2 of the branched glucosyl unit at δ 81.0. Long-range correlations between the anomeric proton of the arabinosyl at δ 5.06 and C-6 of the inner glucosyl at δ 69.0, and between the anomeric proton of the inner glucosyl at δ 5.12 and C-3 of the aglycon at δ 82.5, were also noted. The structure of **2** was thus formulated to be (23*S*)-3*β*-[(*O*-*β*-D-apiofuranosyl-

Table 1. ^{13}C-NMR Spectral Data for **2**, **7**, and **9** in Pyridine-d_5

C	**2**	**7**	**9**
1	35.8	35.6	35.7
2	27.3	27.5	27.5
3	82.5	88.8	88.8
4	48.2	44.4	44.4
5	43.8	51.2	51.7
6	18.8	18.6	18.7
7	26.6	27.3	26.8
8	135.5	133.0	135.1
9	135.0	136.4	134.7
10	36.8	37.2	36.8
11	21.3	20.8	20.9
12	25.4	23.3	24.9
13	49.0	47.6	48.6
14	50.9	58.0	50.6
15	32.1	215.2	31.8
16	39.8	51.9	37.4
17	97.2	91.2	98.6
18	19.4	20.4	18.7
19	19.7	19.4	19.5
20	43.8	43.3	44.0
21	17.3	17.2	18.7
22	36.9	36.8	44.9
23	81.6	81.8	113.4
24	212.5	211.8	44.8
25	32.4	32.2	35.8
26	7.7	7.6	178.9
27	—	—	15.1
28	61.5	23.1	23.1
29	62.7	63.1	63.1
30	26.3	24.1	25.9
1′	105.5	106.0	106.0
2′	75.5	75.4	75.4
3′	78.3	78.2	78.2
4′	72.3	72.5	71.6
5′	75.7	75.9	75.5
6′	69.0	68.4	68.6
1″	101.7	101.2	101.1
2″	77.6	77.2	77.8
3″	71.6	71.3	71.4
4″	67.3	66.6	66.7
5″	64.2	62.5	62.3
1‴	103.6	102.3	102.5
2‴	81.0	77.5	76.9
3‴	88.2	87.7	89.1
4‴	69.4	68.4	69.0
5‴	77.6	77.8	77.7
6‴	62.0	61.9	61.8
1⁗	111.3	101.4	102.1
2⁗	77.6	72.2	72.3
3⁗	79.6	72.5	72.6
4⁗	65.1	74.0	74.1
5⁗	75.2	69.9	69.8
6⁗		18.5	18.6
1′′′′′	104.6	101.0	104.3
2′′′′′	74.9	84.7	74.9
3′′′′′	78.5	78.3	78.5
4′′′′′	72.5	70.7	72.6
5′′′′′	78.3	78.2	78.6
6′′′′′	62.4	62.0	62.7
1′′′′′		106.7	
2′′′′′		76.0	
3′′′′′		78.1	
4′′′′′		70.3	
5′′′′′		78.8	
6′′′′′		62.0	

(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]-17*α*,24-epoxy-28,29-dihydroxy-27-norlanost-8-en-23-one.

Compound **7** was shown to have the molecular formula $C_{64}H_{102}O_{33}$ on the basis of the negative-ion FAB-MS (*m/z* 1397 $[M-H]^-$), ^{13}C-NMR spectrum (64 carbon signals), DEPT data, and elemental analysis. Acid hydrolysis of **7** with 1 M HCl in dioxane–H_2O (1 : 1) gave L-arabinose, D-glucose, and L-rhamnose. The ^{1}H-NMR spectrum of **7** showed signals for six methyl groups at δ 1.72 (s), 1.56 (s), 1.06 (d, J=6.8 Hz), 1.01 (t, J=7.3 Hz), 0.95 (s), and 0.94 (s), characteristic of the 27-norlanostane skeleton, as well as signals for six anomeric protons at δ 6.44 (br s), 5.35 (d, J=7.7 Hz), 5.34 (br s), 5.14 (d, J=7.7 Hz), 5.08 (d, J=7.7 Hz), and 4.97 (d, J=7.8 Hz). These ^{1}H-NMR spectral data of **7** were very similar to those of **5** and suggestive of a 27-norlanostane hexaglycoside closely related to **5**. However, the ^{13}C-NMR spectrum of **7** indicated the presence of two carbonyl groups (δ 215.2, 211.8) in the molecule. One carbonyl group was readily revealed to be located at C-24 by comparison of the ^{13}C-NMR spectrum of **7** with that of **5**, and by long-range correlations from H_2-22 at δ 1.87 (m), H_2-25 at δ 2.45, and Me-26 at δ 1.01 (d, J=7.3 Hz) to the C-24 carbonyl carbon signal at δ 211.8. HMBC correlations from H_2-16 at δ 3.07 and 2.38 (each d, J=19.0 Hz), and Me-30 at δ 1.72 (s) to the δ 215.2 resonance showed the existence of another carbonyl group at C-15. It was ascertained by interpretation of the phase-sensitive nuclear Overhauser effect correlation (NOESY) spectrum that the stereostructure of the aglycon moiety of **7** was the same as that of **4** and **5**. Compound **7** was determined to be (23*S*)-17*α*,23-epoxy-29-hydroxy-3*β*-[(*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)-oxy]-27-norlanost-8-ene-15,24-dione.

Compound **9** was deduced to be $C_{59}H_{94}O_{28}$ from its negative-ion FAB-MS (*m/z* 1249 $[M-H]^-$), ^{13}C-NMR (59 carbon signals), DEPT, and elemental analysis data. The ^{1}H-NMR spectrum of **9** showed signals for six methyl groups at δ 1.54 (s), 1.24 (s), 1.23 (d, J=6.9 Hz), 1.00 (d, J=6.8 Hz), 0.92 (s), and 0.87 (s), and five anomeric protons at δ 6.36 (br s), 5.33 (br s), 5.19 (d, J=7.7 Hz), 5.03 (d, J=7.8 Hz), and 4.96 (d, J=7.8 Hz). The ^{13}C-NMR spectrum of **9** implied the presence of a tetrasubstituted olefinic group [δ 135.1 (C), 134.7 (C)] and a spirolactone group [δ 113.4 (C), 178.9 (C=O)], and was superimposable on that of **10** with resonance for the aglycon moiety. On the other hand, analysis of the ^{13}C-NMR spectrum of **9** allowed identification of the signals for a terminal *β*-D-glucopyranosyl unit, a terminal *α*-L-rhamnopyranosyl unit, a 2,3-disubstituted *β*-D-glucopyranosyl unit, a 2-substituted *α*-L-arabinopyranosyl unit, and a 6-substituted *β*-D-glucopyranosyl unit, and established that the pentaglycoside moiety composed of these monosaccharides was the same as that of **4**. Compound **9** was thus shown to be a new combination of a known triterpene aglycon and sugar, and was characterized as (23*S*,25*R*)-17*α*,23-epoxy-29-hydroxy-3*β*-[(*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]lanost-8-en-23,26-olide.

Table 2. Cytotoxic Activity of **1**—**10** and Etoposide Against HSC-2 Cells

Compound	IC_{50} (*μ*g/ml)
1	>50
2	>50
3	19
4	23
5	10
6	>50
7	>50
8	>50
9	14
10	14
Etoposide	24

In all the isolated compounds, the small $^3J_{H,H}$ value of the anomeric proton and the ^{13}C-NMR shifts of C-1 to C-5 of the C-2-substituted arabinopyranosyl unit implied that it is present as the $^1C_4$ and $^4C_1$ forms in equilibrium with rapid conformational exchange.[4,11]

Compounds **1**—**10** were evaluated for their cytotoxic activity against HSC-2 cells, using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reduction assay procedure (Table 2).[12,13] Compounds **3**—**5**, **9**, and **10** showed cytotoxic activity with LD_{50} values ranging from 10 to 23 *μ*g/ml. Etoposide, to which HSC-2 cells are relatively resistant, had an LD_{50} value of 24 *μ*g/ml. Although **5** was a diastereomer of **6** with regard to the C-23 configuration, only the (23*S*)-isomer (**5**) was cytotoxic. The modification of **5** with a carbonyl group at C-15 led to a significant decrease in the cytotoxicity. The glycoside with an apiofuranosyl moiety did not show any apparent cytotoxicity.

Experimental

Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO A-100 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H-NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA, U.S.A.) mass spectrometer using a dithiothreitol and dithioerythritol (3 : 1) matrix. Elemental analysis was carried out using an Elemental Vario EL elemental analyzer (Hanau, Germany). Diaion HP-20 (Mitsubishi-Kasei, 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 precoated Kieselgel 60 F_{254} (0.25 mm, 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 system comprised of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh CCP PX-8010 controller, a Tosoh UV-8000 or a Tosoh RI-8010 detector, and a Rheodyne injection port with a 2-ml sample loop for preparative HPLC and 20-*μ*l sample loop for analytical HPLC. A Capcell Pak C_{18} UG80 column (10 mm i.d.×250 mm, ODS, 5 *μ*m, Shiseido, Tokyo, Japan) was used for preparative HPLC and a TSK-gel ODS-Prep column (4.6 mm i.d.×250 mm, ODS, 5 *μ*m, Tosoh) was employed for analytical HPLC. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY, U.S.A.); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, U.S.A.); penicillin, streptomycin, and MTT (Sigma, St. Louis, MO, U.S.A.). All other chemicals used were of biochemical reagent grade.

Plant Material 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 for the plant is on file in our laboratory (voucher no. CL-94-003).

Extraction and Isolation The plant material (fresh weight, 3.3 kg) was extracted with hot MeOH. The MeOH extract was concentrated under re-

duced pressure. Column chromatography of the MeOH extract on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH (9 : 1; 6 : 1; 4 : 1; 2 : 1) and finally with MeOH alone gave four fractions (frs. I—IV). Fraction II was chromatographed on ODS silica gel eluted with MeOH–H_2O (4 : 1) into two subfractions (frs. IIa and IIb). Fraction IIa was further separated by a silica gel column eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1; 7 : 4 : 1) to give **3** (45.3 mg) and **4** (253 mg). Fraction IIb was subjected to column chromatography on silica gel eluted with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) and ODS silica gel with MeOH–H_2O (7 : 3), and to preparative HPLC using MeOH–H_2O (4 : 1) to furnish **2** (22.0 mg) and **9** (24.2 mg). Fraction III was subjected to an ODS column eluted with MeOH–H_2O (4 : 1) and divided into three subfractions (frs. IIIa, IIIb, and IIIc). Fraction IIIa was further separated by a silica gel column eluted with $CHCl_3$–MeOH–H_2O (6 : 4 : 1) and preparative HPLC using MeOH–H_2O (3 : 2) to yield **7** (40.0 mg). Fraction IIIb was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (7 : 4 : 1) and ODS silica gel with MeOH–H_2O (4 : 1; 3 : 1) to give **6** (111 mg). Fraction IIIc was subjected to a silica gel column eluted with $CHCl_3$–MeOH–H_2O (7 : 4 : 1), an ODS silica gel column with MeOH–H_2O (3 : 1), and to preparative HPLC using MeCN–H_2O (9 : 11) to afford **5** (80.0 mg) and **10** (146 mg). Fraction IV was separated by column chromatography on ODS silica gel eluted with MeOH–H_2O (4 : 1; 7 : 3) and silica gel with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) to yield pure **8** (20.0 mg) and **1** with a few impurities. The final purification of **1** (20.9 mg) was carried out by preparative HPLC using MeOH–H_2O (7 : 3).

Compound **2**: Amorphous solid, $[\alpha]_D^{26}$ −24.0° (c=0.10, MeOH). FAB-MS (negative mode) m/z: 1223 $[M-H]^-$. *Anal.* Calcd for $C_{57}H_{92}O_{28}\cdot 5H_2O$: C, 52.05; H, 7.82. Found: C, 52.09; H, 7.69. IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2930 and 2880 (CH), 1715 (C=O), 1455, 1370, 1255, 1155, 1070, 1045, 920, and 780. ^{1}H-NMR (pyridine-d_5) δ: 6.24 (1H, d, J=4.0 Hz, H-1‴), 5.15 (1H, d, J=7.9 Hz, H-1‴), 5.12 (1H, d, J=7.6 Hz, H-1′), 5.06 (1H, br s, H-1″), 5.05 (1H, d, J=7.5 Hz, H-1⁗), 1.47 (3H, s, Me-30), 1.08 (3H, s, Me-19), 1.04 (3H, t, J=7.3 Hz, Me-26), 1.02 (3H, d, J=6.4 Hz, Me-21), 0.93 (3H, s, Me-18).

Acid Hydrolysis of 2 A solution of **2** (5.0 mg) in 0.2 M HCl (dioxane–H_2O, 1 : 1, 2 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 fractionated using a Sep-pak C_{18} cartridge (Waters, Milford, MA, U.S.A.) eluted with H_2O–MeOH (4 : 1, 5 ml) followed by MeOH (5 ml) to give a sugar fraction (2.1 mg) and an aglycon fraction (1.5 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 with H_2O–MeCN (4 : 1; 1 : 1, each 5 ml) mixtures as solvents. The H_2O–MeCN (1 : 1) eluate was further passed through a Toyopak IC-SP M cartridge (Tosoh) with EtOH (10 ml) to give a 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative of a monosaccharide,[9] which was then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2 : 3); flow rate, 0.8 ml/min; and detection, UV 230 nm. Derivatives of D-apiose, L-arabinose, D-glucose, and L-rhamnose were detected. t_R (min): 13.28 (derivative of L-arabinose); 13.90 (derivative of D-apiose); 17.74 (derivative of D-glucose); and 19.58 (derivative of L-rhamnose).

Compound **7**: Amorphous solid, $[\alpha]_D^{26}$ −18.0° (c=0.10, MeOH). FAB-MS (negative mode) m/z: 1397 $[M-H]^-$. *Anal.* Calcd for $C_{64}H_{102}O_{33}\cdot 9/2H_2O$: C, 51.92; H, 7.56. Found: C, 51.93; H, 7.82. IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2930 and 2880 (CH), 1720 (C=O), 1450, 1420, 1370, 1310, 1150, 1070, 1040, 915, and 780. ^{1}H-NMR (pyridine-d_5) δ: 6.44 (1H, br s, H-1⁗), 5.35 (1H, d, J=7.7 Hz, H-1⁗⁗), 5.34 (1H, br s, H-1″), 5.14 (1H, d, J=7.7 Hz, H-1‴), 5.08 (1H, d, J=7.7 Hz, H-1⁗⁗), 4.97 (1H, d, J=7.8 Hz, H-1′), 3.07 (1H, d, J=19.0 Hz, H-16a), 2.45 (2H, m, H_2-25), 2.38 (1H, d, J=19.0 Hz, H-16b), 1.87 (2H, m, H_2-22), 1.83 (3H, d, J=6.2 Hz, Me-6⁗), 1.72 (3H, s, Me-30), 1.56 (3H, s, Me-28), 1.06 (3H, d, J=6.8 Hz, Me-21), 1.01 (3H, t, J=7.3 Hz, Me-26), 0.95 (3H, s, Me-18), 0.94 (3H, s, Me-19).

Acid Hydrolysis of 7 Compound **7** (3.8 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was subjected to acid hydrolysis by the same procedures as described for **2** to give a sugar fraction (1.4 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. Derivatives of L-arabinose, D-glucose, and L-rhamnose were detected.

Compound **9**: Amorphous solid, $[\alpha]_D^{24}$ −102.0° (c=0.10, MeOH). FAB-MS (negative mode) m/z: 1249 $[M-H]^-$. *Anal.* Calcd for $C_{59}H_{94}O_{28}\cdot 4H_2O$: C, 53.55; H, 7.77. Found: C, 53.46; H, 8.08. IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2930 and 2880 (CH), 1750 (C=O), 1445, 1410, 1375, 1250, 1150, 1055, 915, and 780. ^{1}H-NMR (pyridine-d_5) δ: 6.36 (1H, br s, H-1⁗), 5.33 (1H, br s, H-1″), 5.19 (1H, d, J=7.7 Hz H-1‴), 5.03 (1H, d, J=7.8 Hz, H-1⁗⁗), 4.96 (1H, d, J=7.8 Hz, H-1′), 1.75 (3H, d, J=6.2 Hz, Me-6⁗), 1.54 (3H, s, Me-28), 1.24 (3H, s, Me-30), 1.23 (3H, d, J=6.9 Hz, Me-27), 1.00 (3H, d, J=6.8 Hz, Me-21), 0.92 (3H, s, Me-19), 0.87 (3H, s, Me-18).

Acid Hydrolysis of 9 Compound **9** (5.9 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was subjected to acid hydrolysis using the same procedures as described for **2** to give a sugar fraction (2.0 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. Derivatives of L-arabinose, D-glucose, and L-rhamnose were detected.

HSC-2 Cell Culture Assay HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM supplemented with 10% heat-inactivated FBS, penicillin 100 units/ml, and streptomycin 100 μg/ml 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, U.S.A.) 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 for 24 h without or with test compounds. They were washed once with PBS and incubated for 4 h with MTT 0.2 mg/ml in DMEM supplemented with 10% FBS. After the medium was removed, the cells were lysed with dimethyl sulfoxide 0.1 ml, and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate using Labsystems Multiskan^R (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10. The LD_{50} value, which reduces the viable cell number by 50%, was determined from the dose-response curve.

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References and Notes

1) Mimaki Y., Ori K., Sashida Y., Nikaido T., Song L.-G., Ohmoto T., *Chem. Lett.*, **1992**, 1999—2000 (1992).
2) Mimaki Y., Ori K., Sashida Y., Nikaido T., Song L.-G., Ohmoto T., *Bull. Chem. Soc. Jpn.*, **66**, 1182—1186 (1993).
3) Mimaki Y., Ori K., Kubo S., Sashida Y., Nikaido T., Song L.-G., Ohmoto T., *Chem. Lett.*, **1992**, 1863—1866 (1992).
4) Kuroda M., Mimaki Y., Ori K., Koshino H., Nukada T., Sakagami H., Sashida Y., *Tetrahedron*, **58**, 6735—6740 (2002).
5) Mimaki Y., Nishino H., Ori K., Kuroda M., Matsui T., Sashida Y., *Chem. Pharm. Bull.*, **42**, 327—332 (1994).
6) Adinolfi M., Barone G., Corsaro M. M., Mangoni L., *Can. J. Chem.*, **66**, 2787—2793 (1988).
7) Sholichin M., Miyahara K., Kawasaki T., *Chem. Pharm. Bull.*, **33**, 1756—1759 (1985).
8) Mimaki Y., Kubo S., Kinoshita Y., Sashida Y., Song L.-G., Nikaido T., Ohmoto T., *Phytochemistry*, **34**, 791—797 (1993).
9) Oshima R., Yamanouchi Y., Kumanotani J., *Carbohydr. Res.*, **107**, 169—176 (1982).
10) Agrawal P. K., *Phytochemistry*, **31**, 3307—3330 (1992).
11) De Tommasi N., Autore G., Bellino A., Pinto A., Pizza C., Sorrentino R., Venturella P., *J. Nat. Prod.*, **63**, 308—314 (2000).
12) Furuya S., Takayama F., Mimaki Y., Sashida Y., Satoh K., Sakagami H., *Anticancer Res.*, **20**, 4189—4194 (2000).
13) Furuya S., Takayama F., Mimaki Y., Sashida Y., Satoh K., Sakagami H., *Anticancer Res.*, **21**, 959—964 (2001).