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27-Norlanostane Glycosides from the Bulbs of *Muscari paradoxum*

Minpei Kuroda,† Yoshihiro Mimaki,*,† Kazutomo Ori,† Hiroshi Sakagami,‡ and Yutaka Sashida†

School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1, Horinouchi, Hachioji, Tokyo 192-0392, Japan, and Department of Dental Pharmacology, Meikai University School of Dentistry, 1-1 Keyaki-dai, Sakado, Saitama 350-0283, Japan

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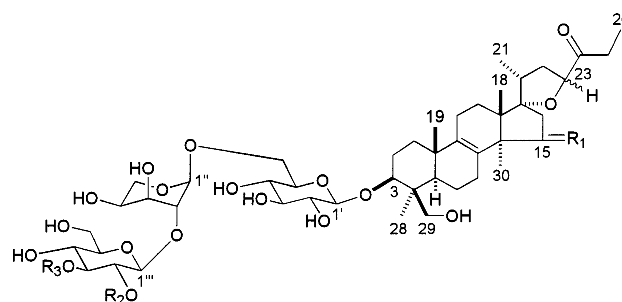
Eight 27-norlanostane glycosides (**1**−**8**), including five new compounds (**3** and **5**−**8**), were isolated from the MeOH extract of the bulbs of *Muscari paradoxum*. The structures of the new compounds were determined on the basis of extensive spectroscopic analysis, including 2D NMR data, and the results of hydrolytic cleavage. The cytotoxic activity of **1**−**8** against HSC-2 human oral squamous cell carcinoma cells is also reported.

Previously, we reported the isolation and structural elucidation of several unique lanosterol oligoglycosides from plants of the subfamily Schilloideae in Liliaceae, such as *Scilla perviana*, *Chionodoxa luciliae*, *C. gigantea*, and *Eucomis bicolor*. Peruvianosides A and B from *S. peruviana* are pentacyclic lanostane triglycosides with a rearranged lanostane side chain moiety.[1,2] Scillasaponin A from *E. bicolor*, B from *S. peruviana*, and C from *C. gigantea* are new lanosterol oligoglycosides with a modified side chain to form a spirolactone ring system.[3] Recently, two new hexaglycosides having a pentacyclic tertranorlanostane skeleton with a γ-lactone ring system, called lucilianosides A and B, were isolated from *C. luciliae*.[4] As part of our continuing search for triterpene glycosides from plants belonging to the Schilloideae, we have now screened the bulbs of *Muscari paradoxum* (Fisch. et. C. A. Mey.) K. Koch, a Schilloideae plant native to western Turkey, and isolated eight 27-norlanostane glycosides (**1**−**8**), including five new compounds (**3** and **5**−**8**). In this paper, we report the structural determination of the new compounds on the basis of extensive spectroscopic analysis, including 2D NMR data, and the results of hydrolytic cleavage. The cytotoxicity of **1**−**8** against HSC-2 human oral squamous cell carcinoma cells is also described.

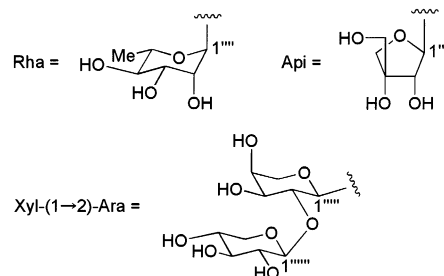
The *n*-BuOH-soluble portion of the MeOH extract of the bulbs was passed through a porous-polymer polystyrene resin (Diaion HP-20) column eluted with 30% MeOH followed by EtOH. The EtOH fraction, in which triterpene glycosides were enriched, was repeatedly subjected to column chromatography over silica gel and octadecylsilanized (ODS) silica gel, as well as to preparative HPLC, to give compounds **1** (30.2 mg), **2** (101 mg), **3** (16.3 mg), **4** (28.0 mg), **5** (5.8 mg), **6** (10.0 mg), **7** (154 mg), and **8** (29.2 mg).

Compounds **1**, **2**, and **4** were identified as (23*S*)-17α,23-epoxy-29-hydroxy-3*β*-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]-27-norlanost-8-en-24-one (**1**),[5] (23*S*)-17α,23-epoxy-29-hydroxy-3*β*-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]-27-norlanost-8-ene-15,24-dione (**2**),[6] and (23*S*)-3*β*-[(*O*-*β*-D-apiofuranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]-17α,23-epoxy-29-hydroxy-27-norlanost-8-ene-15,24-dione (**4**),[7] respectively.

* To whom correspondence should be addressed. Tel: +81-426-76-4577. Fax: +81-426-76-4579. E-mail: mimakiy@ps.toyaku.ac.jp.
† Tokyo University of Pharmacy and Life Science.
‡ Meikai University School of Dentistry.



		R_1	R_2	R_3
1	(23*S*)	H,H	Rha	H
2	(23*S*)	O	Rha	H
3	(23*R*)	O	Rha	H
4	(23*S*)	O	Api	H
5	(23*R*)	O	Api	H
6	(23*S*)	H,H	Rha	Xyl-(1→2)-Ara
7	(23*S*)	O	Rha	Xyl-(1→2)-Ara
8	(23*R*)	O	Rha	Xyl-(1→2)-Ara



Compound **3** was obtained as an amorphous solid, $[\alpha]_D^{28}$ −16.0° (MeOH). Its molecular formula was determined as $C_{52}H_{82}O_{23}$ by the HRESIMS, showing an $[M + H]^+$ peak at *m/z* 1075.5350. The IR spectrum showed a prominent absorption attributable to carbonyl groups at 1730 cm^{-1}, as well as a broad absorption due to hydroxyl groups near 3410 cm^{-1}. The ^{1}H NMR spectrum of **3** showed signals for six methyl groups at δ 1.56 (s), 1.54 (s), 1.13 (d, J = 7.3 Hz), 0.94 (s), 0.93 (s), and 0.86 (d, J = 6.7 Hz), characteristic of the 27-norlanostane skeleton, as well as signals for four anomeric protons at δ 6.38 (br s), 5.35 (d, J = 3.0 Hz), 5.17 (d, J = 7.5 Hz), and 4.97 (d, J = 7.8 Hz). Acid hydrolysis of **3** with 1 M HCl in dioxane−H_2O (1:1) yielded D-glucose, L-arabinose, and L-rhamnose as the carbohydrate moieties, while the labile aglycone was decomposed under acidic conditions. Identification of the monosaccharides, including their absolute configurations, were carried out by direct HPLC analysis of the hydrolysate using a combination of

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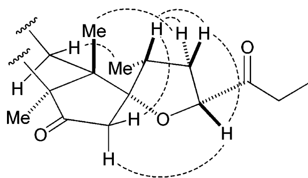

Figure 1. Important NOE correlations of **3**.

RI and optical rotatory (OR) detectors. The ^{1}H and ^{13}C NMR spectra were similar to those of **2** except for the signals due to the Me-21 and Me-30 protons and C-16, C-20, and C-23 carbons, suggesting that **3** was a C-23 epimer of **2**. This was confirmed by NOE correlations as shown in Figure 1. The decisive NOE was between H-16α (δ 2.81) and H-23 (δ 4.41). Thus, the structure of **3** was formulated as (23*R*)-17α,23-epoxy-29-hydroxy-3β-[*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]oxy]-27-norlanost-8-ene-15,24-dione. Compound **3** is believed to be a natural product because **3** was not obtained by refluxing **2** with a mixture of $CHCl_3$−MeOH (1:1) in the presence of silica gel for 5 h.

Compound **5** was analyzed for $C_{51}H_{80}O_{23}$ by the HRESIMS (*m/z* 1061.5188 $[M + H]^+$). The prominent ^{1}H NMR signals arising from the aglycone moiety of **5** at δ 4.43 (dd, *J* = 10.7, 2.6 Hz, H-23), 1.57 (s, Me-30), 1.55 (s, Me-28), 1.13 (t, *J* = 7.2 Hz, Me-26), 0.94 (s, Me-18), 0.93 (s, Me-19), and 0.87 (d, *J* = 6.7 Hz, Me-21) were superimposable on those of **3**, whereas the signals due to the anomeric protons at δ 6.40 (br s), 5.23 (d, *J* = 3.6 Hz), 5.13 (d, *J* = 6.7 Hz), and 4.99 (d, *J* = 7.8 Hz) were coincident with those of **4**. Acid hydrolysis of **5** with 1 M HCl furnished D-apiose, L-arabinose, and D-glucose. Comparison of the ^{13}C NMR spectrum of **5** with those of **3** and **4** and the results of acid hydrolysis, as well as the above ^{1}H NMR data, indicated that **5** was the C-23 epimer of **4**, and the structure was determined as (23*R*)-3β-[(*O*-β-D-apiofuranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-17α,23-epoxy-29-hydroxy-27-norlanost-8-ene-15,24-dione.

Compound **6** was isolated as an amorphous solid with a molecular formula $C_{62}H_{100}O_{30}$, as determined by the data of the HRESIMS exhibiting an $[M + H]^+$ peak at *m/z* 1325.6390. Analysis of the ^{1}H and ^{13}C NMR spectra of **6** indicated that the aglycone of **6** was the same as that of **1**. The ^{1}H NMR spectrum of **6** displayed signals for six anomeric protons at δ 6.13 (1H, br s), 5.28 (1H, d, *J* = 7.7 Hz), 5.26 (1H, d, *J* = 3.5 Hz), 5.18 (1H, d, *J* = 7.6 Hz), 5.17 (1H, d, *J* = 6.2 Hz), and 4.98 (1H, d, *J* = 7.9 Hz), as well as one three-proton doublet at δ 1.73 (*J* = 6.2 Hz), which was associated with the methyl carbon at δ 18.7, implying that one of the six monosaccharide moieties of **6** was 6-deoxyhexose. Acid hydrolysis of **6** with 1 M HCl in dioxane−H_2O (1:1) gave L-arabinose, D-glucose, L-rhamnose, and D-xylose. The ^{1}H−^{1}H COSY and TOCSY experiments allowed the sequential assignments of the signals from H-1 to H_2-5, H_2-6, or Me-6 of each monosaccharide. The HMQC spectrum was used to associate the protons with the relevant carbon resonances. Comparison of the carbon shifts with those of reference methyl glycosides,[8,9] taking into account the proton spin-coupling constants, the known effects of *O*-glycosylation, and the results of acid hydrolysis, indicated that **6** contained a terminal β-D-xylopyranosyl unit (Xyl), a terminal α-L-rhamnopyranosyl unit (Rha), two α-L-arabinopyranosyl units glycosylated at C-2 (Ara and Ara′), a C-2 and C-3 branched β-D-glucopyranosyl unit (Glc′), and a β-D-glucopyranosyl unit glycosylated at C-6 (Glc). The ^{13}C NMR shifts and the proton spin-coupling constant (*J* = 6.2 Hz) of Ara′ showed that it was present as the $^{4}C_1$ form with an α-configurated anomeric center, whereas the ^{13}C NMR shifts and the small $^{3}J_{H-1,H-2}$ value (3.5 Hz) of Ara were indicative of the predominance of the $^{1}C_4$ conformation with the α-anomeric configuration.[10] The sugar sequences of **6** were determined by the following HMBC correlations. The anomeric proton of Xyl at δ 5.28 showed a long-range correlation with C-2 of Ara′ at δ 78.9, of which the anomeric proton at δ 5.17, in turn, exhibited a correlation with C-3 of Glc′ at δ 85.9. The anomeric protons of Rha at δ 6.13 and Glc′ at δ 5.18 showed HMBC correlations with C-2 of Glc′ at δ 78.6 and C-2 of Ara at δ 77.7, respectively. Furthermore, long-range correlations between the anomeric proton of Ara at δ 5.26 and C-6 of Glc at δ 68.6 and between the anomeric proton of Glc at δ 4.98 and C-3 of the aglycone at δ 88.9 were observed. Accordingly, the structure of **6** was established as (23*S*)-17α,23-epoxy-29-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-27-norlanost-8-en-24-one. The branched hexaglycoside of **6** has not been reported as a sugar moiety in either triterpene or steroidal saponins.

Compound **7** had a molecular formula of $C_{62}H_{98}O_{31}$ from its HRESIMS (*m/z* 1339.6180 $[M + H]^+$). The ^{1}H NMR spectrum of **7** showed signals for six methyl groups at δ 1.69 (s), 1.54 (s), 1.05 (d, *J* = 6.7 Hz), 1.02 (d, *J* = 7.3 Hz), 0.94 (s), and 0.93 (s), and six anomeric protons at δ 6.12 (br s), 5.27 (d, *J* = 7.9 Hz), 5.26 (d, *J* = 3.5 Hz), 5.18 (d, *J* = 7.5 Hz), 5.16 (d, *J* = 6.0 Hz), and 4.97 (d, *J* = 7.8 Hz). The ^{13}C NMR spectrum of **7** indicated the presence of a tetrasubstituted olefinic group (δ 136.5 and 133.0) and two carbonyl groups (δ 215.2 and 211.7) and was superimposable on those of **2** and **4** as far as the resonances for the aglycone moiety are concerned. Analysis of the ^{13}C NMR spectrum of **7** allowed identification of the signals for a terminal β-D-xylopyranosyl unit, a terminal α-L-rhamnopyranosyl unit, two α-L-arabinopyranosyl units glycosylated at C-2, a C-2 and C-3 branched β-D-glucopyranosyl unit, and a β-D-glucopyranosyl unit glycosylated at C-6, and established that the hexaglycoside moiety composed of these monosaccharides was the same as that of **6**. The structure of **7** was thus characterized as (23*S*)-17α,23-epoxy-29-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-27-norlanost-8-ene-15,24-dione.

Compound **8** exhibited a molecular formula of $C_{62}H_{98}O_{31}$ on the basis of the HRESIMS (*m/z* 1139.6182 $[M + H]^+$). Analysis of the ^{1}H and ^{13}C NMR spectra of **8** and comparison with those of **7** implied that **8** differed from **7** only in the C-23 configuration. NOE correlations between H-16α (δ 2.82) and H-23 (δ 4.42) confirmed the 23*R* configuration. The structure of **8** was assigned as (23*R*)-17α,23-epoxy-29-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-27-norlanost-8-ene-15,24-dione.

Compounds **1**−**8** were evaluated for their cytotoxic activity against HSC-2 cells. Compounds **1**, **2**, **4**, **6**, and **7** showed cytotoxic activity (IC_{50}: **1**, 32 μM; **2**, 63 μM; **4**, 6.2 μM; **6**, 7.3 μM; **7**, 19 μM), among which the activity of **4** and **6** was much more potent than that of etoposide (IC_{50}: 41 μM) used as a positive control. It is notable that the corresponding 23*R*-isomers **3**, **5**, and **8** of **2**, **4**, and **7** did

Table 1. ^{13}C NMR Data for **3**, **5**, and **6**−**8** in C_5D_5N

	3	**5**	**6**	**7**	**8**
1	35.7	35.7	35.8	35.6	35.6
2	27.5	27.5	27.5	27.3	27.4
3	88.9	88.9	88.9	88.8	88.9
4	44.4	44.4	44.4	44.4	44.4
5	51.4	51.4	51.8	51.2	51.4
6	18.7	18.7	18.7	18.6	18.7
7	27.5	27.5	26.9	27.4	27.5
8	133.1	133.1	135.5	133.0	133.1
9	136.6	136.6	134.7	136.5	136.6
10	37.3	37.3	36.8	37.2	37.3
11	20.8	20.8	21.1	20.7	20.8
12	23.3	23.3	25.3	23.2	23.3
13	47.4	47.4	48.9	47.6	47.4
14	58.1	58.1	50.9	58.0	58.0
15	215.0	215.0	32.1	215.2	215.0
16	49.4	49.4	39.8	51.9	49.4
17	91.2	91.2	97.1	91.2	91.1
18	20.4	20.4	19.3	20.4	20.4
19	19.3	19.3	19.5	19.3	19.3
20	41.0	41.0	43.7	43.3	41.0
21	18.4	18.4	17.3	17.1	18.4
22	36.0	36.0	36.9	36.8	36.0
23	79.8	80.3	81.6	81.8	80.3
24	212.3	212.3	212.5	211.7	212.3
25	32.0	32.0	32.4	32.3	32.0
26	7.8	7.8	7.7	7.6	7.8
27	−	−	−	−	−
28	23.2	23.2	23.2	23.1	23.1
29	63.2	63.2	63.2	63.1	63.1
30	24.5	24.5	26.4	24.1	24.5
1′	106.1	106.1	106.1	106.1	106.1
2′	75.4	75.4	75.4	75.4	75.4
3′	78.3	78.2	78.3	78.2	78.2
4′	71.4	71.3	72.6	72.4	72.4
5′	75.5	75.7	75.5	75.6	75.6
6′	68.6	68.9	68.6	68.6	68.7
1″	100.9	101.4	101.2	101.3	101.3
2″	78.3	78.3	77.7	77.6	77.6
3″	71.4	72.1	71.6	71.6	71.8
4″	66.3	66.9	66.9	66.9	67.8
5″	62.1	62.2	63.1	63.1	63.1
1‴	103.1	103.6	102.6	102.5	102.5
2‴	77.6	79.8	78.6	78.7	78.7
3‴	79.4	79.6	85.9	85.8	85.8
4‴	72.7	72.5	68.8	68.8	68.8
5‴	78.2	78.5	77.8	77.8	77.8
6‴	62.1	63.3	61.8	61.8	61.9
1⁗	101.9	111.1	102.2	102.2	102.1
2⁗	72.3	77.9	72.2	72.2	72.2
3⁗	72.6	80.4	72.7	72.6	72.5
4⁗	74.2	65.9	74.0	73.9	74.0
5⁗	69.7	75.4	70.0	69.9	69.9
6⁗	18.7		18.7	18.7	18.7
1′′′′′			102.3	102.3	102.1
2′′′′′			78.9	78.9	78.9
3′′′′′			73.7	73.7	73.7
4′′′′′			68.3	68.3	68.2
5′′′′′			65.9	65.9	65.9
1′′′′′′			105.6	105.6	105.6
2′′′′′′			75.1	75.1	75.0
3′′′′′′			78.2	78.1	78.0
4′′′′′′			70.9	70.8	70.9
5′′′′′′			67.4	67.3	67.3

not show any apparent cytotoxic activity even at the sample concentration of 100 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 (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 spectrometer (500 MHz for ^{1}H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ-values with reference to tetramethylsilane (TMS) as internal standard. ESIMS data were obtained on a Micromass LCT mass spectrometer (Manchester, UK). Daiaion 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 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 with 10% H_2SO_4 followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh) or a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port. A Capcell Pak C_{18} UG120 column (10 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for preparative HPLC. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), penicillin, and streptomycin (Sigma, St. Louis, MO). All other chemicals used were of biochemical reagent grade.

Table 2. ^{1}H NMR Data for the Sugar Moiety of **6** in C_5D_5N

position		^{1}H	*J* (Hz)	position		^{1}H	*J* (Hz)
Glc′	1	4.98 d	7.9	Rha⁗	1	6.13 br s	
	2	3.98 dd	8.5, 7.9		2	4.82 br d	3.3
	3	4.18 dd	8.5, 8.5		3	4.61 dd	9.2, 3.3
	4	4.16 dd	8.5, 8.5		4	4.26 dd	9.2, 9.2
	5	4.03 ddd	8.5, 4.6, 3.8		5	4.83 dq	9.2, 6.2
	6a	4.53 dd	11.7, 3.8		6	1.73 d	6.2
	6b	4.29 dd	11.7, 4.6				
Ara″	1	5.26 d	3.5	Ara′′′′′	1	5.17 d	6.2
	2	4.67 dd	6.7, 3.5		2	4.67 dd	8.0, 6.2
	3	4.65 dd	6.7, 6.7		3	4.25 m	
	4	4.51 m			4	4.26 m	
	5a	4.35 br d	11.2		5a	4.35 dd	11.4, 4.0
	5b	3.89 dd	11.2, 3.5		5b	3.68 br d	11.4
Glc‴	1	5.18 d	7.6	Xyl′′′′′′	1	5.28 d	7.7
	2	4.15 dd	8.5, 7.6		2	4.19 dd	9.0, 7.7
	3	4.09 dd	8.5, 8.5		3	4.03 dd	9.0, 9.0
	4	4.05 dd	8.5, 8.5		4	4.11 ddd	10.5, 9.0, 5.6
	5	3.58 m			5a	4.25 dd	10.5, 5.6
	6a	4.20 dd	11.0, 3.0		5b	3.54 dd	10.5, 10.5
	6b	4.12 m					

Plant Material. The bulbs of *M. paradoxum* were purchased from a nursery in Heiwaen, Nara, Japan. The bulbs were cultivated, and the flowered plant was identified by one of the authors (Y.S.). A voucher specimen of the plant has been deposited in our laboratory (95-003-MP).

Extraction and Isolation. The plant material (fresh weight, 3.0 kg) was extracted with hot MeOH. The MeOH extract was concentrated under reduced pressure, and the viscous concentrate was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble phase was passed through a Diaion HP-20 column eluted with 30% MeOH followed by EtOH. Column chromatography of the EtOH eluate portion on ODS silica gel and elution with a stepwise gradient mixture of MeOH−H_2O (1:2; 1:1; 2:1), and finally with MeOH alone, gave five fractions (I−V). Fraction II was chromatographed on silica gel eluted with $CHCl_3$−MeOH−H_2O (30:10:1) and divided into three further subfractions (IIa, IIb, and IIc). Fraction IIb was subjected to column chromatography on silica gel eluted with $CHCl_3$−MeOH−H_2O (30:10:1) and preparative HPLC using MeOH−H_2O (16:7) to yield **2** (101 mg), **3** (16.3 mg), **4** (28.0 mg), and **5** (5.8 mg). Fraction IIc was subjected to silica gel column chromatography eluted with $CHCl_3$−MeOH−H_2O (30:10:1), ODS silica gel column chromatography with MeCN−H_2O (2:5), and preparative HPLC using MeOH−H_2O (5:6) to give **7** (154 mg) and **8** (29.2 mg). Fraction III was further separated by column chromatography on silica gel eluted with $CHCl_3$−MeOH−H_2O (20:10:1) into three subfractions (IIIa,

IIIb, and IIIc). Fraction IIIa was subjected to a silica gel column eluted with $CHCl_3$−MeOH−H_2O (40:10:1) and preparative HPLC using MeOH−H_2O (16:5) to yield **1** (30.2 mg). Compound **6** (10.0 mg) was isolated from fraction IIIc by subjecting it to a silica gel column eluted with $CHCl_3$−MeOH−H_2O (40:10:1) and preparative HPLC using MeOH−H_2O (16:7).

Compound 3: amorphous solid; $[\alpha]_D^{28}$ −16.0° (*c* 0.10, MeOH); IR ν_{max} (film) 3410 (OH), 2930 and 2900 (CH), 1730 (C=O), 1050 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 6.38 (1H, br s, H-1′′′′), 5.35 (1H, d, J = 3.0 Hz, H-1′′), 5.17 (1H, d, J = 7.5 Hz, H-1′′′), 4.97 (1H, d, J = 7.8 Hz, H-1′), 4.41 (1H, dd, J = 10.6, 2.5 Hz, H-23), 2.83 (1H, dq, J = 18.4, 7.2 Hz, H-25a), 2.81 (1H, d, J = 19.1 Hz, H-16α), 2.70 (1H, dq, J = 18.4, 7.2 Hz, H-25b), 2.51 (1H, m, H-12α), 2.36 (1H, d, J = 19.1 Hz, H-16β), 2.22 (1H, m, H-22a), 2.02 (1H, m, H-20), 1.93 (1H, m, H-22b), 1.75 (3H, d, J = 6.1 Hz, Me-6′′′′), 1.59 (1H, m, H-12β), 1.56 (3H, s, Me-30), 1.54 (3H, s, Me-28), 1.13 (3H, t, J = 7.3 Hz, Me-26), 0.94 (3H, s, Me-18), 0.93 (3H, s, Me-19), 0.86 (3H, d, J = 6.7 Hz, Me-21); HRESIMS *m/z* 1075.5350 [M + H]$^+$ (calcd for $C_{52}H_{83}O_{23}$, 1075.5324).

Acid Hydrolysis of 3. A solution of **3** (5.0 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 95 °C for 1 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 Diaion HP-20 eluted with H_2O−MeOH (3:2) followed by Me_2CO−EtOH (1:1) to give a sugar fraction (1.5 mg). The sugar fraction was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA) and a Toyopak IC-SP-M cartridge (Tosoh), 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 (17:3); flow rate, 0.9 mL/min; detection, RI and OR. Identification of L-rhamnose, L-arabinose, and D-glucose present in the sugar fraction was carried out by the comparison of their retention times and polarities with those of authentic samples; t_R (min) 7.60 (L-rhamnose, negative polarity), 9.22 (L-arabinose, positive polarity), 14.88 (D-glucose, positive polarity).

Compound 5: amorphous solid; $[\alpha]_D^{28}$ −2.0° (*c* 0.10, MeOH); IR ν_{max} (film) 3410 (OH), 2940 and 2880 (CH), 1730 (C=O), 1060, 1030, 1000 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 6.40 (1H, br s, H-1′′′′), 5.23 (1H, d, J = 3.6 Hz, H-1′′), 5.13 (1H, d, J = 6.7 Hz H-1′), 4.99 (1H, d, J = 7.8 Hz, H-1′′′), 4.43 (1H, dd, J = 10.7, 2.6 Hz, H-23), 2.85 (1H, dq, J = 18.3, 7.2 Hz, H-25a), 2.82 (1H, d, J = 19.0 Hz, H-16α), 2.70 (1H, dq, J = 18.3, 7.2 Hz, H-25b), 2.53 (1H, m, H-12α), 2.36 (1H, d, J = 19.0 Hz, H-16β), 2.21 (1H, m, H-22a), 2.00 (1H, m, H-20), 1.94 (1H, m, H-22b), 1.59 (1H, m, H-12β), 1.57 (3H, s, Me-30), 1.55 (3H, s, Me-28), 1.13 (3H, t, J = 7.2 Hz, Me-26), 0.94 (3H, s, Me-18), 0.93 (3H, s, Me-19), 0.87 (3H, d, J = 6.7 Hz, Me-21); HRESIMS *m/z* 1061.5188 [M + H]$^+$ (calcd for $C_{51}H_{81}O_{23}$, 1061.5168).

Acid Hydrolysis of 5. A solution of **5** (2.0 mg) in 0.2 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 95 °C for 30 min under an Ar atmosphere, and the reaction mixture was treated as described for **3**. HPLC analysis of the sugar fraction (0.7 mg) under the same conditions as for **3** showed the presence of D-apiose, L-arabinose, and D-glucose in the sugar fraction; t_R (min) 7.11 (D-apiose, positive polarity), 9.22 (L-arabinose, positive polarity), 14.86 (D-glucose, positive polarity).

Compound 6: amorphous solid; $[\alpha]_D^{28}$ −54.0° (*c* 0.10, MeOH); IR ν_{max} (film) 3377 (OH), 2933 and 2882 (CH), 1713 (C=O), 1076, 1047 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 1.56 (3H, s, Me-28), 1.53 (3H, s, Me-30), 1.07 (3H, d, J = 7.3 Hz, Me-26), 1.03 (3H, d, J = 6.6 Hz, Me-21), 0.94 (3H, s, Me-19), 0.91 (3H, s, Me-18); HRESIMS *m/z* 1325.6390 [M + H]$^+$ (calcd for $C_{62}H_{101}O_{30}$, 1325.6376).

Acid Hydrolysis of 6. Compound **6** (1.7 mg) was subjected to acid hydrolysis as described for **3** to give a sugar fraction (0.3 mg). HPLC analysis of the sugar fraction under the same conditions as for **3** showed the presence of L-rhamnose, L-arabinose, D-xylose, and D-glucose in the sugar fraction; t_R (min) 8.66 (L-rhamnose, negative polarity), 10.38 (L-arabinose, positive polarity), 10.84 (D-xylose, positive polarity), 17.97 (D-glucose, positive polarity).

Compound 7: amorphous solid; $[\alpha]_D^{28}$ −22.0° (*c* 0.10, MeOH); IR ν_{max} (film) 3400 (OH), 2980, 2950, and 2880 (CH), 1730 (C=O), 1050 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 6.12 (1H, br s, H-1′′′′), 5.27 (1H, d, J = 7.9 Hz, H-1′′′′′′), 5.26 (1H, d, J = 3.5 Hz, H-1′′), 5.18 (1H, d, J = 7.5 Hz, H-1′′′), 5.16 (1H, d, J = 6.0 Hz, H-1′′′′′), 4.97 (1H, d, J = 7.8 Hz, H-1′), 3.05 (1H, d, J = 19.0 Hz, H-16α), 2.45 (1H, q, J = 7.3 Hz, H-25), 2.39 (1H, d, J = 19.0 Hz, H-16β), 1.72 (3H, d, J = 6.2 Hz, Me-6′′′′), 1.69 (3H, s, Me-30), 1.54 (3H, s, Me-28), 1.05 (3H, d, J = 6.7 Hz, Me-21), 1.02 (3H, d, J = 7.3 Hz, Me-26), 0.94 (3H, s, Me-18), 0.93 (3H, s, Me-19); HRESIMS *m/z* 1339.6180 [M + H]$^+$ (calcd for $C_{62}H_{99}O_{31}$, 1339.6169).

Acid Hydrolysis of 7. Compound **7** (2.0 mg) was subjected to acid hydrolysis as described for **3** to give a sugar fraction (0.6 mg). HPLC analysis of the sugar fraction under the same conditions as for **3** showed the presence of L-rhamnose, L-arabinose, D-xylose, and D-glucose; t_R (min) 7.50 (L-rhamnose, negative polarity), 9.15 (L-arabinose, positive polarity), 9.66 (D-xylose, positive polarity), 14.79 (D-glucose, positive polarity).

Compound 8: amorphous solid, $[\alpha]_D^{28}$ −12.0° (*c* 0.10, MeOH); IR ν_{max} (film) 3400 (OH), 2930 and 2980 (CH), 1730 (C=O), 1050 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 6.11 (1H, d, J = 1.0 Hz, H-1′′′′), 5.30 (1H, d, J = 7.9 Hz, H-1′′′′′′) 5.28 (1H, d, J = 3.2 Hz, H-1′′), 5.24 (1H, d, J = 7.5 Hz, H-1′′′), 5.18 (1H, d, J = 6.1 Hz, H-1′′′′′), 4.97 (1H, d, J = 7.8 Hz, H-1′), 4.43 (1H, m, H-23), 2.84 (1H, dq, J = 18.4, 7.2 Hz, H-25a), 2.82 (1H, d, J = 19.1 Hz, H-16α), 2.70 (1H, dq, J = 18.4, 7.2 Hz, H-25b), 2.50 (1H, m, H-12α), 2.36 (1H, d, J = 19.1 Hz, H-16β), 2.24 (1H, m, H-22a), 2.01 (1H, m, H-20), 1.92 (1H, dd, J = 13.0, 2.5 Hz, H-22b), 1.66 (3H, d, J = 6.2 Hz, Me-6′′′′), 1.60 (1H, dd, J = 12.8, 9.9 Hz, H-12β), 1.51 (3H, s, Me-30), 1.50 (3H, s, Me-28), 1.13 (3H, d, J = 7.3 Hz, Me-26), 0.93 (3H, s, Me-18), 0.90 (3H, s, Me-19), 0.88 (3H, d, J = 6.8 Hz, Me-21); HRESIMS *m/z* 1339.6182 [M + H]$^+$ (calcd for $C_{62}H_{99}O_{31}$, 1339.6169).

Acid Hydrolysis of 8. Compound **8** (2.5 mg) was subjected to acid hydrolysis as described for **3** to give a sugar fraction (1.1 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **3** showed the presence of L-rhamnose, L-arabinose, D-xylose, and D-glucose; t_R (min) 8.68 (L-rhamnose, negative polarity), 10.40 (L-arabinose, positive polarity), 10.86 (D-xylose, positive polarity), 18.00 (D-glucose, positive polarity).

HSC-2 Cell Culture Assay. HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM supplemented with 10% heat-inactivated FBS 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) and incubated for 24 h. After washing once with phosphate-buffered saline (PBS), they were treated for 24 h without or with test compounds. The cells 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 of 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.[11,12]

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