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Lanosterol and tetranorlanosterol glycosides from the bulbs of *Muscari paradoxum*

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Abstract

Three tetranorlanostane glycosides, named lucilianosides C–E, and three lanostane glycosides, named scillasaponins E–G, together with a known lanostane glycoside, were isolated from the MeOH extract of *Muscari paradoxum* (Liliaceae) bulbs, as confirmed by detailed analysis of their ^{1}H, ^{13}C, and two-dimensional NMR spectroscopic data, and by the results of hydrolytic cleavage. The isolated compounds were evaluated for their cytotoxic activity against HSC-2 human oral squamous cell carcinoma cells.

Keywords: *Muscari paradoxum*; Liliaceae; Tetranorlanostane glycosides; Lucilianosides C–E; Lanostane glycosides; Scillasaponins E–G; Cytotoxic activity; HSC-2 cells

1. Introduction

Previous phytochemical studies have revealed that plants belonging to the subfamily Schilloideae in Liliaceae such as *Scilla peruviana*, *Chionodoxa gigantea*, *C. luciliae*, and *Eucomis bicolor* contain a variety of triterpenes oligoglycosides with the lanostane skeleton, some of which are unique in structure. Examples include peruvianosides A and B from *Scilla peruviana*, which are pentacyclic lanostane triglycosides with a rearranged lanostane side chain moiety (Mimaki et al., 1993b), and scillasaponin A from *Eucomis bicolor*, B from *S. peruviana*, and C from *Chionodoxa gigantea* which are new lanosterol oligoglycosides with the side chain modified to form a spirolactone ring system (Mimaki et al., 1992). Recently, two hexaglycosides having a pentacyclic tetranorlanostane skeleton with a γ-lactone ring system, called lucilianosides A and B, were isolated and characterized from *Chionodoxa luciliae* (Kuroda et al., 2002). Other research groups have also recently isolated several lanosterol and norlanosterol glycosides from *C. luciliae* (Barone et al., 1993; Adinolfi et al., 1993) and *S. scilloides* (Lee et al., 2002). As part of a continuing search for triterpene glycosides from plants belonging to the Schilloideae, the bulbs of *Muscari paradoxum* (Fisch. et C.A. Mey.) K. Koch, a Schilloideae plant native to western Turkey, were studied, leading to the isolation of three new tetranorlanostane glycosides, named lucilianosides C–E (**1**–**3**), and three new lanostane glycosides, named scillasaponins E–G (**4**–**6**), together with a known lanostane glycoside (**7**). This paper mainly reports the structural determination 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. The current work is the second chemical investigation of *M. parodoxum*, next to a qualitative HPLC analysis of anthocyanins and anthocyanidines in the flowers (Mori et al., 2002) (Schemes 1 and 2).

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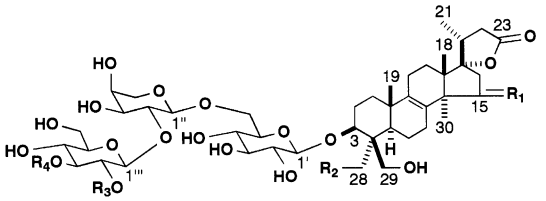
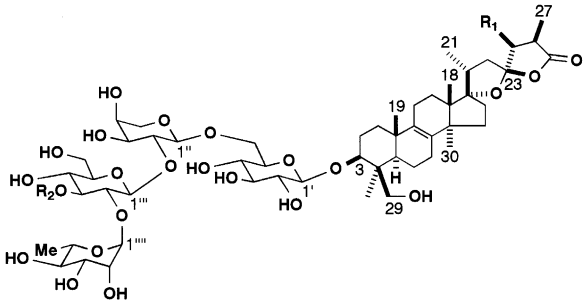
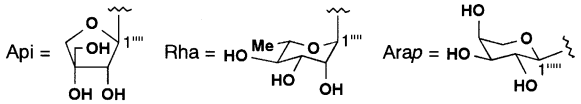


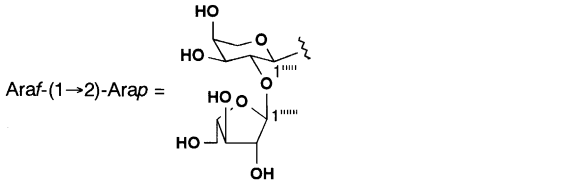
	R_1	R_2	R_3	R_4
1	H,H	HO	Api	H
2	O	H	Rha	Ara*p*
3	O	H	Rha	Ara*f*-(1→2)-Ara*p*

Scheme 1.



	R_1	R_2
4	H	Ara*p*
5	H	Ara*f*-(1→2)-Ara*p*
6	HO	Ara*f*-(1→2)-Ara*p*
7	H	H





Scheme 2.

2. Results and discussion

A concentrated *n*-BuOH-soluble portion of the MeOH extract of *M. paradoxum* bulbs (fresh weight of 3 kg) was passed through a porous-polymer polystyrene resin (Diaion HP-20) column, and the EtOH eluate fraction was subjected to column chromatography (CC) using silica gel and octadecylsilanized (ODS) silica gel, as well as to preparative reversed-phase HPLC, to yield **1** (7.2 mg), **2** (45.2 mg), **3** (15.6 mg), **4** (31.8 mg), **5** (13.7 mg), **6** (24.8 mg), and **7** (87.6 mg). Compound **7** ($C_{58}H_{92}O_{27}$) was identified by its mass, IR, ^{1}H NMR, and ^{13}C NMR spectral data as (23*S*,25*R*)-17α,23-epoxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]lanost-8-en-23,26-olide (Mimaki et al., 1994).

Lucilianoside C (**1**) was isolated as an amorphous solid, $[\alpha]_D$ −48.0° (MeOH). The positive-ion HR-ESIMS of **1** showed an accurate $[M+Na]^+$ ion at *m/z* 1043.4664 in accordance with an empirical molecular formula of $C_{48}H_{76}O_{23}$, which was supported by the ^{13}C NMR spectrum and various DEPT spectral data. The IR spectrum showed an absorption attributable to an ester carbonyl group at 1744 cm^{-1}, as well as a broad absorption due to hydroxyl groups near 3346 cm^{-1}. The ^{1}H NMR spectrum of **1** (C_5D_5N) was typical of a triterpene glycoside, showing signals for three tertiary methyl groups at δ 1.13, 1.02, and 0.87 (each *s*), a secondary methyl group at δ 1.00 (*d*, *J* = 6.7 Hz), an axial-oriented methine proton geminal to a glycosyloxy group at δ 4.50 (*dd*, *J* = 11.9, 4.9 Hz), and four anomeric protons at δ 6.42 (*d*, *J* = 1.9 Hz), 5.19 (*br s*), 5.14 (*d*, *J* = 7.5 Hz), and 5.12 (*d*, *J* = 7.0 Hz). Furthermore, signals for two hydroxymethyl groups linked to the same quaternary carbon at δ 4.93 and 4.37 (each *d*, *J* = 11.4 Hz), and 4.61 and 3.95 (each *d*, *J* = 11.4 Hz), and a deshielded methylene group adjacent to an ester carbonyl group at δ 2.75 (*dd*, *J* = 16.5, 6.3 Hz) and 2.04 (*br d*, *J* = 16.5 Hz) were observed as the characteristic ^{1}H NMR signals for **1**. These ^{1}H NMR spectroscopic data and comparison of the ^{13}C NMR spectrum of **1** (Table 1) with that of 3β,28,29-trihydroxy-24,25,26,27-tetranorlanost-8-en-17α,23-olide 3-*O*-hexaglycoside (lucilianoside B) (Kuroda et al., 2002) indicated that the structure of the aglycone moiety of **1**, including its stereochemistry, was identical that of lucilianoside B. On the other hand, the ^{13}C NMR signals due to the tetraglycoside moiety of **1** were similar to those of the known compound **7**, except for the terminal sugar unit. Instead of the signals for a rhamnosyl group, the five signals of a pentose were observed at δ 111.1 (CH), 77.9 (CH), 80.4 (C), 75.4 (CH_2), and 65.9 (CH_2), which, except for the δ 80.4 resonance, were associated with the one-bond coupled proton signals at δ 6.42 (*d*, *J* = 1.9 Hz), 4.81 (*d*, *J* = 1.9 Hz), 4.82 and 4.37 (each *d*, *J* = 9.3 Hz), and 4.27 (2H, *br s*), respectively, by the HMQC spectrum. Acid hydrolysis of **1** with 0.2 M HCl in dioxane–H_2O (1:1) gave D-apiose, L-arabinose, and D-glucose as the carbohydrate components, while the labile aglycone was decomposed under acidic conditions. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate, with detection being carried out by using an optical rotation (OR) detector. These data implied that the terminal monosaccharide in **1** is β-D-apiofuranose (Kitagawa et al., 1989). The glycosidic linkage of the apiosyl unit to C-2 of the inner glucosyl group was confirmed by a long-range correlation from the H-1 signal of the apiosyl at δ 6.42 to C-2 of the inner glucosyl at δ 79.7 in the HMBC spectrum. Long-range

Table 1
^{13}C NMR spectral data for **1**–**6** in C_5D_5N

Position	1	2	3	4	5	6
1	35.7	35.5	35.5	35.7	35.7	35.7
2	27.2	27.3	27.3	27.5	27.5	27.5
3	82.1	88.8	88.8	88.9	88.9	88.9
4	48.2	44.3	44.3	44.4	44.4	44.4
5	43.4	51.6	51.1	51.7	51.7	51.7
6	18.6	18.6	18.6	18.7	18.7	18.7
7	26.5	27.2	27.2	26.8	26.8	26.8
8	134.8	132.6	132.6	135.1	135.1	135.1
9	135.8	136.8	136.9	134.7	134.7	134.7
10	36.7	37.2	37.2	36.8	36.8	36.8
11	20.9	20.5	20.5	21.0	21.0	21.0
12	24.7	22.7	22.7	24.9	24.9	25.0
13	49.0	47.6	47.6	48.7	48.7	48.8
14	50.8	58.0	57.9	50.7	50.7	50.8
15	31.6	213.0	213.0	31.8	31.8	31.9
16	39.1	50.7	50.7	37.4	37.4	37.7
17	97.9	91.8	91.7	98.6	98.6	99.2
18	18.0	17.5	19.2	18.7	18.7	18.7
19	19.5	19.2	19.2	19.5	19.5	19.5
20	41.7	41.0	41.0	44.0	44.0	43.8
21	17.6	19.2	17.5	18.7	18.7	18.6
22	39.1	38.7	38.7	44.9	44.9	38.5
23	176.7	176.2	176.2	113.4	113.4	116.9
24				44.8	44.8	77.4
25				35.8	35.8	41.2
26				178.9	178.9	178.7
27				15.1	15.1	8.9
28	61.1	23.1	23.1	23.1	23.1	23.1
29	62.6	63.1	63.1	63.2	63.2	63.1
30	25.6	23.6	23.6	26.0	26.0	26.0
1′	105.5	106.1	106.1	106.1	106.1	106.1
2′	75.4	75.4	75.4	75.4	75.4	75.4
3′	78.3	78.2	78.3	78.2	78.3	78.2
4′	71.2	72.6	72.9	72.7	72.9	72.9
5′	75.5	75.6	75.7	75.5	75.6	75.6
6′	68.9	68.7	68.8	68.7	68.8	68.8
1″	101.3	101.2	101.4	101.1	101.4	101.4
2″	79.6	77.8	77.9	77.8	77.8	77.8
3″	72.1	71.7	71.8	71.6	71.7	71.7
4″	66.9	66.8	67.0	66.8	66.9	66.8
5″	63.2	62.9	63.0	63.2	63.1	63.1
1‴	103.6	102.5	102.7	102.5	102.6	102.6
2‴	79.7	77.6	79.4	77.5	79.3	79.3
3‴	78.6	87.5	85.3	87.5	85.4	85.4
4‴	72.6	69.0	68.9	69.0	68.9	68.9
5‴	78.2	77.8	77.7	77.8	77.7	77.8
6‴	62.0	61.8	61.8	61.8	61.8	61.8
1⁗	111.1	102.3	102.5	102.3	102.8	102.8
2⁗	77.9	72.2	71.9	72.2	71.9	71.9
3⁗	80.4	72.7	72.5	72.6	72.5	72.5
4⁗	75.4	74.1	74.0	74.1	74.0	74.0
5⁗	65.9	69.9	70.3	69.9	70.3	70.2
6⁗		18.7	18.7	18.6	18.7	18.7
1′′′′′		105.3	102.8	105.3	102.8	102.8
2′′′′′		72.2	76.2	72.2	76.1	76.1
3′′′′′		74.5	72.4	74.5	72.4	72.4
4′′′′′		69.3	68.0	69.3	68.0	68.0
5′′′′′		67.6	65.6	67.6	65.7	65.7
1′′′′′′			110.0		110.0	110.0
2′′′′′′			81.8		81.7	81.7
3′′′′′′			78.7		78.7	78.7
4′′′′′′			87.3		87.3	87.3
5′′′′′′			62.5		62.5	62.5

correlations from H-1 of the inner glucosyl at δ 5.12 to C-2 of the arabinosyl at δ 79.6, H-1 of the arabinosyl at δ 5.19 to C-6 of the glucosyl at δ 68.9, and H-1 of the glucosyl at δ 5.14 to C-3 of the aglycone at δ 82.1 were also noted. Accordingly, the structure of **1** was determined as 3β-[(*O*-β-D-apiofuranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-28,29-dihydroxy-24,25,26,27-tetranorlanost-8-en-17α,23-olide.

Lucilianoside D (**2**) was obtained as an amorphous solid with a molecular formula $C_{54}H_{84}O_{27}$, as determined by the positive-ion HR-ESIMS exhibiting an $[M+Na]^+$ ion at *m*/*z* 1187.5088 in conjunction with the ^{13}C NMR and DEPT spectral data. The spectral features of **2** were essentially analogous with those of **1**; however, the ^1H NMR signals for the four tertiary methyl groups at δ 1.55, 1.38, 0.93, and 0.91 (each *s*), and a hydroxymethyl group at δ 4.40 and 3.62 (each *d*, *J* = 11.2 Hz), along with a secondary methyl group at δ 1.05 (*d*, *J* = 6.8 Hz) and a methylene group adjacent to an ester carbonyl group at δ 2.88 (*dd*, *J* = 16.8, 6.6 Hz) and 2.16 (*br d*, *J* = 16.8 Hz) suggested that the aglycone of **2** is more similar to that of lucilianoside A (3β,29-dihydroxy-24,25,26,27-tetranorlanost-8-en-17α,23-olide 3-*O*-hexaglycoside) (Kuroda et al., 2002) than that of lucilianoside B. Comparison of the ^{13}C NMR spectrum of **2** with that of lucilianoside A showed their close similarity to the signals due to the aglycone, except for the presence of one carbonyl carbon signal at δ 213.0 and the lack of a methylene carbon signal. The three-proton singlet signal at δ 1.38, which was assigned to Me-30 by long-range correlations with C-8 at δ 132.6 and C-14 at δ 58.0, showed a $^3J_{C,H}$ correlation with the carbonyl carbon signal at δ 213.0. The isolated methylene proton signals at δ 3.03 and 2.81 (each *d*, *J* = 19.3 Hz) were exclusively assigned to H_2-16 by the observa-

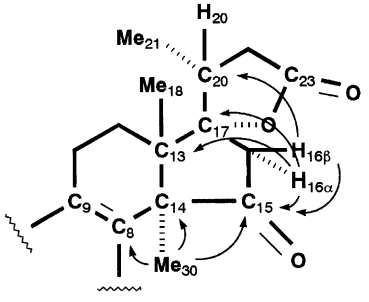


Fig. 1. Partial HMBC correlations of aglycone moiety of **2**.

tion of HMBC correlations from δ 3.03 to C-13 at δ 47.6 and C-17 at δ 91.8, and δ 2.81 to C-20 at δ 41.0, and H_2-16 also exhibited long-range correlations with the carbonyl carbon (Fig. 1). These data gave ample evidence for the location of the carbonyl group at C-15. The ring junction patterns and stereochemistry at C-3, C-17, and C-21 were shown to be the same as those of lucilianoside A by interpretation of the phase-sensitive NOESY spectrum (Fig. 2) (Kuroda et al., 2002). The ^{1}H NMR spectrum of **2** showed five anomeric proton signals at δ 6.23 (*br s*), 5.31 (*d*, *J* = 3.6 Hz), 5.21 (*d*, *J* = 7.7 Hz), 4.97 (*d*, *J* = 7.5 Hz), and 4.85 (*d*, *J* = 7.3 Hz), and acid hydrolysis of **2** gave L-arabinose, D-glucose, and L-rhamnose. When the ^{13}C NMR spectral shifts of the sugar moiety of **2** with those of **7**, a set of additional five signals due to a pentose were observed at δ 105.3 (CH), 74.5 (CH), 72.2 (CH), 69.3 (CH), and 67.6 (CH_2). The C-1 signal at δ 105.3 was associated with the H-1 signal at δ 4.85 by the HMQC spectrum. Analysis of the ^{1}H-^{1}H COSY spectrum revealed that the spin system starting with H-1 extended to three oxymethine and one oxymethylene groups, and this sugar was identified as α-L-arabinopyranose due to the axial–axial couplings between H-1 and H-2, and between H-2 and H-3, and the axial–equatorial relationship between H-3 and H-4 (Table 2). The H-1 signal of the additional arabinosyl showed a long-range correlation with C-3 of the 2,3-branched glucosyl attached at C-2 of the inner arabinosyl at δ 87.5. An HMBC correlation between H-1 of the terminal rhamnosyl at δ 6.23 and C-2 of the 2,3-branched glucosyl at δ 77.6 was also observed. All of these data for **2** were consistent with the structure 3β-[(*O*-α-L-arabinopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-29-hydroxy-15-oxo-24,25,26,27-tetranorlanost-8-en-17α,23-olide.

Lucilianoside E (**3**) was shown to have the molecular formula $C_{59}H_{92}O_{31}$ on the basis of the positive-ion HR-ESIMS (*m*/*z* 1297.5690 $[M+H]^+$), ^{13}C NMR, and DEPT spectral data. The ^{1}H and ^{13}C NMR spectra allowed the identification of its aglycone as being the same as that of **2**, and the ^{1}H NMR spectrum displayed six anomeric proton signals at δ 6.05 (*br s*), 5.97 (*br s*), 5.28 (*d*, *J* = 3.7 Hz), 5.21 (*d*, *J* = 6.9 Hz), 5.18 (*d*, *J* = 5.8 Hz), and 4.98 (*d*, *J* = 7.8 Hz). Acid hydrolysis of **3** afforded L-arabinose, D-glucose, and L-rhamnose. These results and comparison of the ^{13}C NMR spectrum of **3** with that of **2** suggested that **3** structurally corresponded to **2** with one more pentose unit present. The C-1 signal of the pentose appeared at δ 110.0 (CH), which corresponded to the H-1 signal at δ 6.05. Analysis of the ^{1}H–^{1}H COSY spectrum starting from H-1 revealed the spin-coupling correlations and multiplet patterns of the pentose originating protons. Since every proton coupled to the vicinal proton(s) with small coupling constant(s), the pentose was shown to have a furanose form. The protons assigned as H-1–H_2-5 were correlated to the one-bond coupled carbons using the HMQC spectrum, resulting in the assignment of C-1–C-5 as δ 110.0 (CH), 81.8 (CH), 78.7 (CH), 87.3 (CH), and 62.5 (CH_2) in order. Thus, the additional pentose was revealed to be α-L-arabinofuranose (Agrawal, 1992). A $^3J_{C,H}$ correlation was observed from H-1 of the arabinofuranosyl at δ 6.05 to the δ 76.2 resonance assignable to C-2 of the arabinopyranosyl group, of which the H-1 signal at δ 5.18 had an HMBC correlation with C-3 of the 2,3-branched glucosyl unit at δ 85.3. The structure of **3** was elucidated as 3β-[(*O*-α-L-arabinofuranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-29-hydroxy-15-oxo-24,25,26,27-tetranorlanost-8-en-17α,23-olide.

Scillasaponin E (**4**) was analyzed for $C_{58}H_{92}O_{27}$ by the positive-ion HR-ESIMS (*m*/*z* 1243.5730 $[M+Na]^+$), ^{13}C NMR, and DEPT spectral data. The ^{1}H NMR spectrum of **4** showed signals for six methyl groups at δ 1.54 (*s*), 1.24 (*s*), 1.23 (*d*, *J* = 7.2 Hz), 1.01 (*d*, *J* = 6.8 Hz), 0.94 (*s*), and 0.88 (*s*), and five anomeric protons at δ 6.22 (*br s*), 5.30 (*d*, *J* = 3.5 Hz), 5.19 (*d*, *J* = 7.6 Hz), 4.94 (*d*, *J* = 7.8 Hz), and 4.84 (*d*, *J* = 7.3 Hz). The ^{13}C NMR spectrum of **4** implied the presence of a tetrasubstituted olefinic group [δ 135.1 (C) and 134.7 (C)] and a spirolactone group [δ 113.4 (C) and 178.9 (C=O)], and was superimposable to that of **7** with resonance for the aglycone moiety. On the other hand, analysis of the ^{13}C NMR spectrum of **4** allowed the identification of the signals for a terminal α-L-arabinopyranosyl 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 **2**. Compound **4** was characterized as (23*S*,25*R*)-3β-[(*O*-α-L-arabinopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-17α,23-epoxy-29-hydroxylanost-8-en-23,26-olide.

Scillasaponin F (**5**: $C_{63}H_{100}O_{31}$) was shown to be a lanostane glycoside whose aglycone moiety was the same as that of **4** and **7**. On comparison of the ^{13}C NMR spectrum of the sugar moiety of **5** with that of **4**,

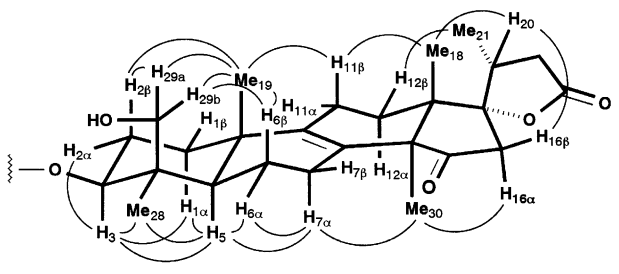


Fig. 2. NOE correlations of aglycone moiety of **2**.

Table 2
^{1}H NMR spectral data for aglycone moiety of **2** and saccharide moiety of **2** and **3** in C_5D_5N

	2			**2**				**3**	
Position	^{1}H	*J* (Hz)	Position	^{1}H	*J* (Hz)	Position	^{1}H	J (Hz)
1 eq	1.63 *br d*	13.0	Glc′ 1	4.97 *d*	7.5	Glc′ 1	4.98 *d*	7.8
ax	1.12 *ddd*	13.0, 13.0, 3.1	2	3.98 *t*-like	8.5	2	3.99 *t*-like	8.3
2 eq	2.28 *br dd*	12.9, 3.1	3	4.17 *dd*	9.0, 9.0	3	4.19 *t*-like	8.8
ax	1.99 *q*-like	12.9	4	4.16		4	4.18 *t*-like	8.8
3	3.59 *dd*	12.1, 4.7	5	4.02		5	4.03	
4	–		6a	4.56 *dd*	10.3, 3.6	6a	4.56 *dd*	12.0, 3.0
5	1.23 *br d*	12.8	b	4.27		b	4.30 *dd*	12.0, 5.0
6 α	1.87 *br dd*	12.3, 7.6	Ara″ 1	5.31 *d*	3.6	Ara″ 1	5.28 *d*	3.7
β	1.47 *m*		2	4.69 *dd*	5.7, 3.6	2	4.67 *dd*	5.5, 3.7
7 α	2.79 *dd*	16.5, 6.0	3	4.67 *br d*	5.7	3	4.64 *dd*	5.5, 3.3
β	2.51 *m*		4	4.03	7.1, 3.4	4	4.48 *m*	
8	–		5a	4.37 *dd*	11.2, 7.1	5a	4.36 *dd*	11.2, 7.2
9	–		b	3.91 *dd*	11.2, 3.4	b	3.87 *dd*	11.2, 3.3
10	–		Glc‴ 1	5.21 *d*	7.7	Glc‴ 1	5.21 *d*	6.9
11 α	2.11		2	4.13 *t*-like	8.2	2	4.12 *t*-like	8.0
β	1.95 *m*		3	4.05 *dd*	8.9, 8.9	3	4.10 *t*-like	8.3
12 α	2.27		4	4.03		4	4.03 *t*-like	8.3
β	1.57 *br dd*	13.1, 10.2	5	3.61 *m*		5	3.62	
13	–		6a	4.23		6a	4.23 *dd*	11.6, 1.7
14	–		b	4.17		b	4.16 *dd*	11.6, 4.9
15	–		Rha⁗ 1	6.23 *br s*		Rha⁗ 1	5.97 *br s*	
16 α	3.03 *d*	19.3	2	4.83 *br d*	3.3	2	4.90 *br d*	3.2
β	2.81 *d*	19.3	3	4.63 *dd*	9.3, 3.3	3	4.59 *dd*	9.4, 3.2
17	–		4	4.28 *dd*	9.3, 9.3	4	4.29 *dd*	9.4, 9.4
18	0.93 *s*		5	4.87		5	4.78 *dq*	9.4, 6.2
19	0.91 s		6	1.74 *d*	6.1	6	1.75 *d*	6.2
20	2.46 *m*		Ara⁗ 1	4.85 *d*	7.3	Ara⁗ 1	5.18 *d*	5.8
21	1.05 *d*	6.8	2	4.14 *dd*	9.4, 7.3	2	4.55 *dd*	7.5, 5.8
22 α	2.88 *dd*	16.8, 6.6	3	4.03 *dd*	9.4, 9.4	3	4.19 *br d*	7.5
β	2.16 *brd*	16.8	4	4.23		4	4.22	
23	–		5a	4.24		5a	4.35 *dd*	11.8, 5.1
28	1.55 *s*		b	3.75 *br d*	11.4	b	3.68 *br d*	11.8
29 α	4.40 *d*	11.2				Ara⁗ 1	6.05 *br s*	
β	3.62					2	5.03 *br d*	3.3
30	1.38 *s*					3	4.73 *dd*	5.3, 3.3
						4	4.90	
						5a	4.22 *br d*	11.9
						b	4.08 *br d*	11.9

five signals corresponding to an α-L-arabinofuranosyl unit appeared and the C-2 signal of the terminal arabinopyranosyl moiety was shifted downfield by 3.9 ppm. Furthermore, the ^{13}C NMR signals arising from the sugar moiety of **5** were in excellent agreement with those of **3**. The structure of **5** was formulated as (23*S*,25*R*)-3β-[(*O*-α-L-arabinofuranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-*O*α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-17α,23-epoxy-29-hydroxylanost-8-en-23,26-olide.

Scillasaponin G (**6**) was deduced as $C_{63}H_{100}O_{32}$ from its positive-ion HR-ESIMS (*m*/*z* 1369.6240 $[M+H]^+$), ^{13}C NMR spectral, and elemental analysis data. The spectral properties of **6** were very similar to those of **5** and were suggestive of a lanostane hexaglycoside closely related to **5**. However, the molecular formula of **6** was greater by one oxygen atom than that of **5**, and differences were recognized only in the NMR signals arising from the spirolactone moiety (E- and F-ring parts) between **5** and **6**. Comparison of the ^{1}H and ^{13}C NMR spectra of **6** with those of scillasaponin D, which is a lanostane tetraglycoside of the spirolactone type with a (24*S*)-hydroxyl group [Mimaki et al., 1993a], allowed the identification of the aglycone of **6** as being the same as that of the reference compound. The structure of **6** was shown to be (23*S*,24*S*,25*R*)-3β-[(*O*-α-L-arabinofuranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyr;anosyl)oxy]-17α,23-epoxy-24,29-dihydroxylanost-8-en-23,26-olide.

In all the isolated compounds, the small $^3J_{H,H}$ value of the anomeric proton and the ^{13}C-NMR shifts of C-1–

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 the rapid conformational exchange (De Tommasi et al., 2000; Kuroda et al., 2002).

Compounds **1**–**6** are new triterpene oligoglycosides based upon the lanostane skeleton, and this is the second account on the isolation of the tetranorlanostane glycosides from natural sources (Kuroda et al., 2002).

The isolated compounds were evaluated for their cytotoxic activity against HSC-2 cells. Although the tetranorlanostane glycosides (**1**–**3**) did not show any apparent cytotoxicity against HSC-2 cells even at a sample concentration of 100 μg/ml, the lanostane glycosides with a spirolactone ring system (**4**–**7**) exhibited cytotoxic activity with IC_{50} values ranging from 6.3 to 59 μg/ml, when etoposide, to which HSC-2 cells are relatively resistant had an IC_{50} value of 24 μg/ml (Table 3). The spirolactone moiety in **4**–**7** is considered to contribute to the appearance of their cytotoxic activity.

3. Experimental

3.1. General

ESIMS: Micromass LCT (Manchester, UK). NMR (ppm, *J* Hz): Bruker AM-500 (500 MHz for ^1H NMR, Karlsruhe, Germany). CC: Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan); silica gel (Fuji-Silysia Chemical, Aichi, Japan); ODS silica gel (Nacalai Tesque, Kyoto, Japan). TLC: precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP-18 $F_{254}S$ (0.25 mm thick, Merck). Preparative HPLC: a Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010) equipped with an RI detector (Tosoh RI-8010, Tokyo, Japan) and a Capcell Pak C_{18} UG80 column (10 mm i.d. × 250 mm, ODS, 5 μm, Shiseido, Tokyo, Japan). Cell culture and assay for cytotoxic activity: Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY, USA); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, USA); penicillin and streptomycin sulfate (Meiji-Seika, Tokyo, Japan); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA). All other chemicals used were of biochemical reagent grade.

Table 3
Cytotoxic activity of compounds **1**–**7** and etoposide against HSC-2 cells

Compound	IC_{50} (μg/ml)
1	>100
2	>100
3	>100
4	6.3
5	23
6	59
7	42
etoposide	24

3.2. Plant material

The bulbs of *M. paradoxum* were purchased from a nursery in Heiwaen, Nara, Japan. The bulbs were cultivated, and the flowering plant was identified by one of the authors (Y.S.). A voucher specimen has been deposited in the laboratory (voucher No. MP-95-003, Laboratory of Medicinal Plant Science).

3.3. Extraction and isolation

The fresh bulbs of *M. paradoxum* (3 kg) were 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 MeOH–H_2O (3:7) followed by EtOH. CC 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 applied to silica gel CC eluted with $CHCl_3$-MeOH-H_2O (30:10:1) and was divided into three subfractions (IIa, IIb, and IIc). Fraction IIa was sequentially subjected to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (30:10:1), ODS silica gel CC with MeCN–H_2O (5:13), and from preparative HPLC using MeOH–H_2O (3:2) to give **1** (7.2 mg). Fraction IIc was separated by silica gel CC eluted with $CHCl_3$–MeOH–H_2O (30:10:1), ODS silica gel CC with MeCN-H_2O (2:5), and preparative HPLC using MeOH–H_2O (5:6) to give **2** (45.2 mg) and **3** (15.6 mg). Fraction III was divided by CC 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 CC on silica gel CC eluted with $CHCl_3$–MeOH–H_2O (40:10:1) and preparative HPLC using MeOH–H_2O (16:5) to give **7** (87.6 mg). Fraction IIIb was subjected to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (30:10:1) and ODS silica gel with MeCN–H_2O (5:8) to give a mixture of two compounds, which were separated by preparative HPLC using MeOH–H_2O (16:5) to afford **4** (31.8 mg) and **5** (13.7 mg). Compound **6** (24.8 mg) was isolated from fraction IIIc by subjecting it to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (40:10:1) and preparative HPLC using MeOH–H_2O (16:7).

3.4. Lucilianoside C (*1*)

Amorphous solid. $[\alpha]_D^{27}$ −48.0° (MeOH; *c* 0.10). HR-ESIMS (positive-mode) *m/z*: 1043.4664 $[M+Na]^+$ (1043.4674 requires for $C_{48}H_{76}O_{23}Na$); IR ν_{max} (film)

cm^{-1}: 3346 (OH), 2937, 2925 and 2880 (CH), 1744 (C=O), 1450, 1414, 1373, 1312, 1154, 1073, 1044, 1010, 950, 919, 865; ^1H NMR (C_5D_5N): δ 6.42 (1H, *d*, *J* = 1.9 Hz, H-1‴′), 5.19 (1H, *br s*, H-1″), 5.14 (1H, *d*, *J* = 7.5 Hz, H-1′), 5.12 (1H, *d*, *J* = 7.0 Hz, H-1‴), 4.93 and 4.37 (each 1H, *d*, *J* = 11.4 Hz, H_2-28), 4.61 and 3.95 (each 1H, *d*, *J* = 11.4 Hz, H_2-29), 4.50 (1H, *dd*, *J* = 11.9, 4.9 Hz, H-3), 2.75 (1H, *dd*, *J* = 16.5, 6.3 Hz, H-22a), 2.04 (1H, *br d*, *J* = 16.5 Hz, H-22b), 1.13 (3H, *s*, Me-30), 1.02 (3H, *s*, Me-19), 1.00 (3H, *d*, *J* = 6.7 Hz, Me-21), 0.87 (3H, *s*, Me-18); for ^{13}C NMR, see Table 1.

3.5. Acid hydrolysis of 1

A solution of **1** (2 mg) in 0.2 M HCl (dioxane-H_2O, 1:1, 2 ml) was heated at 95 °C for 30 min under Ar. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and subjected to Diaion HP-20 CC eluted with H_2O–MeOH (3:2) followed by Me_2CO-EtOH (1:1) to give a sugar fraction (0.8 mg) and an aglycone fraction (0.4 mg). TLC analysis of the aglycone fraction showed that it contained several unidentified artifactual sapogenols. The sugar fraction was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) and a Toyopak IC-SP-M cartridge (Tosoh), which was then analyzed by HPLC under the following conditions, showing the presence of D-apiose, L-arabinose, and D-glucose. Column: Capcell Pak NH_2 UG80 (4.6 mm i.d.×250 mm, 5 μm, Shiseido, Japan); detector: Shodex OR-2 (Showa-Denko, Tokyo, Japan); solvent: MeCN–H_2O (17:3); flow rate: 0.9 ml/min. R_t (min): 7.91 (D-apiose, positive optical rotation); 10.45 (L-arabinose, positive optical rotation); 17.72 (D-glucose, positive optical rotation).

3.6. Lucilianoside D (2)

Amorphous solid. $[\alpha]_D^{26}$ −32.0° (MeOH; *c* 0.10). HR-ESIMS (positive-mode) *m/z*: 1187.5088 $[M+Na]^+$ (1187.5097 requires for $C_{54}H_{84}O_{27}Na$); IR ν_{max} (film) cm^{-1}: 3376 (OH), 2935 (CH), 1793 and 1735 (C=O), 1415, 1378, 1292, 1259, 1198, 1149, 1072, 1043, 933, 869; ^1H NMR: Table 2; ^{13}C NMR: Table 1.

3.7. Acid hydrolysis of 2

Compound **2** (3.0 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction (0.6 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose. R_t (min): 8.64 (L-rhamnose, negative optical rotation); 10.37 (L-arabinose, positive optical rotation); 17.55 (D-glucose, positive optical rotation).

3.8. Lucilianoside E (3)

Amorphous solid. $[\alpha]_D^{26}$ −38.5° (MeOH; *c* 0.13). HR-ESIMS (positive-mode) *m/z*: 1297.5699 $[M+H]^+$ (1297.5690 requires for $C_{59}H_{93}O_{31}$); IR ν_{max} (film) cm^{-1}: 3375 (OH), 2932 and 2879 (CH), 1794 and 1733 (C=O), 1401, 1378, 1281, 1258, 1197, 1148, 1118, 1073, 1044, 1013, 933, 870; ^1H NMR (C_5D_5N): δ 6.05 (1H, *br s*, H-1⁗′), 5.97 (1H, *br s*, H-1‴′), 5.28 (1H, *d*, *J* = 3.7 Hz, H-1″), 5.21 (1H, *d*, *J* = 6.9 Hz, H-1‴), 5.18 (1H, *d*, *J* = 5.8 Hz, H-1⁗′), 4.98 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.40 and 3.63 (each 1H, *d*, *J* = 11.2 Hz, H_2-29), 3.59 (1H, *dd*, *J* = 11.5, 4.7 Hz, H-3), 3.02 and 2.81 (each 1H, *d*, *J* = 19.3 Hz, H_2-16), 2.88 (1H, *dd*, *J* = 16.8, 6.7 Hz, H-22a), 2.16 (1H, *br d*, *J* = 16.8 Hz, H-22b), 1.75 (3H, *d*, *J* = 6.2 Hz, Me-6‴′), 1.55 (3H, *s*, Me-28), 1.37 (3H, *s*, Me-30), 1.05 (3H, *d*, *J* = 6.7 Hz, Me-21), 0.93 (3H, *s*, Me-18), 0.90 (3H, *s*, Me-19); for ^{13}C NMR see Table 1.

3.9. Acid hydrolysis of 3

Compound **3** (1 mg) was subjected to acid hydrolysis as for **1** to give a sugar fraction (0.3 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose.

3.10. Scillasaponin E (4)

Amorphous solid. $[\alpha]_D^{27}$ −22.0° (MeOH; *c* 0.10). HR-ESIMS (positive-mode) *m/z*: 1243.5730 $[M+Na]^+$ (1243.5726 requires for $C_{58}H_{92}O_{27}Na$); IR ν_{max} (film) cm^{-1}: 3410 (OH), 2930 and 2880 (CH), 1765 (C=O), 1450, 1370, 1250, 1210, 1150, 1050, 910, 880, 860, 830, 800, 770; ^1H NMR (C_5D_5N): δ 6.22 (1H, *br s*, H-1‴′), 5.30 (1H, *d*, *J* = 3.5 Hz, H-1″), 5.19 (1H, *d*, *J* = 7.6 Hz, H-1′), 4.94 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.84 (1H, *d*, *J* = 7.3 Hz, H-1⁗′), 2.66 (1H, *dd*, *J* = 13.4, 6.6 Hz, H-22a), 1.73 (3H, *d*, *J* = 6.2 Hz, Me-6‴′), 1.72 (1H, *br d*, *J* = 13.4 Hz, H-22b), 1.54 (3H, *s*, Me-28), 1.24 (3H, *s*, Me-30), 1.23 (3H, *d*, *J* = 7.2 Hz, Me-27), 1.01 (3H, *d*, *J* = 6.8 Hz, Me-21), 0.94 (3H, *s*, Me-19), 0.88 (3H, *s*, Me-18); for ^{13}C NMR see Table 1.

3.11. Acid hydrolysis of 4

Compound **4** (2 mg) was subjected to acid hydrolysis as for **1** to give a sugar fraction (0.9 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose.

3.12. Scillasaponin F (5)

Amorphous solid. $[\alpha]_D^{28}$ −40.0° (MeOH; *c* 0.10). HR-ESIMS (positive-mode) *m/z*: 1353.6323 $[M+H]^+$

(1353.6327 requires for $C_{63}H_{101}O_{31}H$). (Found: C, 53.80; H, 7.54. Calc. for $C_{63}H_{100}O_{31}\cdot 3H_2O$: C, 53.76; H, 7.59%); IR ν_{max} (film) cm^{-1}: 3420 (OH), 2910 and 2880 (CH), 1760 (C=O), 1445, 1360, 1245, 1210, 1160, 1040, 905, 890, 850, 800, 770; ^1H NMR (C_5D_5N): δ 6.05 (1H, *br s*, H-1‴″), 5.98 (1H, *br s*, H-1″), 5.29 (1H, *d*, J=3.3 Hz, H-1″), 5.20 (1H, *d*, J=6.8 Hz, H-1‴), 5.18 (1H, *d*, J=5.8 Hz, H-1⁗), 4.98 (1H, *d*, J=7.8 Hz, H-1′), 1.77 (3H, *d*, J=6.1 Hz, Me-6‴), 1.55 (3H, *s*, Me-28), 1.24 (3H, *s*, Me-30), 1.23 (3H, *d*, J=7.3 Hz, Me-27), 1.00 (3H, *d*, J=6.7 Hz, Me-21), 0.92 (3H, *s*, Me-19), 0.87 (3H, *s*, Me-18); for ^{13}C NMR see Table 1.

3.13. Acid hydrolysis of 5

Compound **5** (1.5 mg) was subjected to acid hydrolysis as for **1** to give a sugar fraction (0.9 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose.

3.14. Scillasaponin G (6)

Amorphous solid. $[\alpha]_D^{28}$ −36.0° (MeOH; *c* 0.10). HR-ESIMS (positive-mode) *m/z*: 1369.6240 $[M+H]^+$ (1369.6276 requires for $C_{63}H_{101}O_{32}H$). (Found: C, 53.80; H, 7.51. Calc. for $C_{63}H_{100}O_{32}\cdot 2H_2O$: C, 53.84; H, 7.46%); IR ν_{max} (film) cm^{-1}: 3410 (OH), 2930 and 2900 (CH), 1760 (C=O), 1450, 1370, 1250, 1050, 930, 900, 850, 830, 800, 780; ^1H NMR (C_5D_5N): δ 6.04 (1H, *br s*, H-1⁗″), 5.96 (1H, *br s*, H-1‴″), 5.29 (1H, *d*, J=3.3 Hz, H-1″), 5.19 (1H, *d*, J=6.8 Hz, H-1‴), 5.16 (1H, *d*, J=5.8 Hz, H-1⁗″), 4.97 (1H, *d*, J=7.8 Hz, H-1′), 4.40 (1H, *d*, J=4.8 Hz, H-24), 3.32 (1H, *m*, H-25), 2.69 (1H, *br d*, J=14.1 Hz, H-22b), 2.53 (1H, *dd*, J=14.1, 6.4 Hz, H-22a), 1.74 (3H, *d*, J=6.1 Hz, Me-6‴), 1.55 (3H, *s*, Me-28), 1.50 (3H, *d*, J=7.2 Hz, Me-27), 1.28 (3H, *s*, Me-30), 1.09 (3H, *d*, J=6.8 Hz, Me-21), 0.94 (3H, *s*, Me-19), 0.90 (3H, *s*, Me-18); for ^{13}C NMR, see Table 1.

3.15. Acid hydrolysis of 6

Compound **6** (1.6 mg) was subjected to acid hydrolysis as for **1** to give a sugar fraction (0.7 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose.

3.16. Cell culture and assay for cytotoxic activity

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 sulfate 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 PBS, they were treated for 24 h with or without test compounds. They were washed once with PBS and incubated for 4 h with 0.2 mg/ml MTT in DMEM medium 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^R (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10. The IC_{50} value, which reduces the viable cell number by 50%, was determined from the dose-response curve (Furuya et al., 2000; Furuya et al., 2001).

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