Myrseguinosides A—E, Five New Glycosides from the Fruits of *Myrsine seguinii*

Katsuyoshi MATSUNAMI,[a] Hideaki OTSUKA,*,[a] and Yoshio TAKEDA[b]

[a] *Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University; 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8553, Japan: and* [b] *Faculty of Pharmaceutical Sciences, Yasuda Women's University; 6–13–1 Yasuhigashi, Asaminami-ku, Hiroshima 731–0153, Japan.*

Received June 28, 2011; accepted July 25, 2011; published online August 1, 2011

Chemical investigation of the 1-BuOH soluble fraction of the dried fruits of *Myrsine seguinii* (Myrsinaceae) led to the isolation of five new glycosides, named myrseguinosides A—E (1—5), together with eight known compounds (6—13). The absolute structures of the new glycosides were elucidated by spectroscopic and chemical analyses to be a monoterpene glucoside (1), two flavonol glycosides (2, 3), and two oleanane-type triterpene saponins (4, 5). Myrseguinosides B (2), D (4), and E (5) exhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity and growth inhibitory activity toward human cancer cells, respectively.

Key words *Myrsine seguinii*; Myrsinaceae; myrseguinoside; radical scavenging activity; cytotoxicity

In our continuing studies on the chemical constituents of *Myrsine seguinii* H. LÉVEILLÉ (syn. *Rapanea neriifolia*) (Myrsinaceae) collected in Okinawa, five new glycosides along with eight known compounds were isolated from the dried fruits of this plant. In our previous research, flavonol glycosides, seguinosides A—K and myrsinionosides A—E, were isolated from the leaves of the title plant.[1—4] Hirota *et al.* also reported the isolation of benzoic acid derivatives, myrsinoic acids A—F, as anti-inflammatory compounds from the fresh leaves and twigs of this plant.[5,6] However, no phytochemical investigation on the fruits of *M. seguinii* have been performed so far. This paper deals with structural elucidation of the five new compounds, named myrseguinosides A—E (**1**—**5**) from the fruits of this plant. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and human cancer cell growth inhibitory activities of these compounds are also discussed.

Results and Discussion

Air-dried fruits (670 g) of *M. seguinii* were extracted with MeOH three times by maceration. The combined methanolic extracts were evaporated under reduced pressure to yield a viscous gummy material. The MeOH extract was then subjected to various kinds of column chromatography to yield 13 compounds (**1**—**13**) (Fig. 1).

Compound **1** was obtained as a colorless amorphous powder and its elemental composition was determined to be $C_{16}H_{28}O_7$ on the basis of high-resolution (HR)-electrospray ionization (ESI)-time-of-flight (TOF)-MS (*m*/*z*=355.1722 $[M+Na]^+$). The strong IR absorption at 3382 cm^{-1} indicated the presence of multiple hydroxy functionalities. In the ^1H- and ^{13}C-NMR spectra, an anomeric proton signal at δ_H 4.35 (1H, d, *J*=8 Hz), and six oxygenated carbon signals at δ_C 102.2, 78.3, 78.0, 75.2, 72.0 and 63.1 indicated the presence of a β-glucopyranosyl group (Table 1). The remaining ten

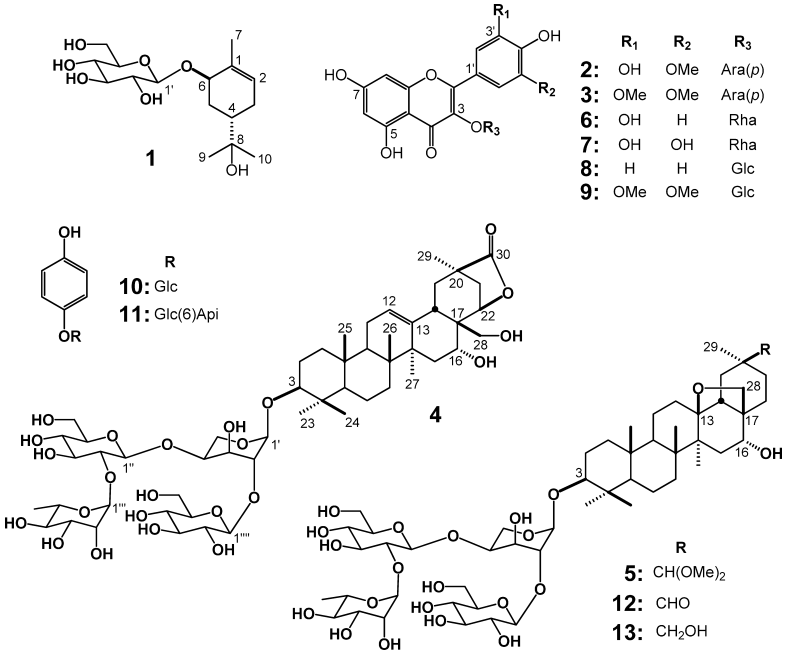

Fig. 1. Structures of the Isolated Compounds

* To whom correspondence should be addressed. e-mail: hotsuka@hiroshima-u.ac.jp

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1** (600, 150 MHz, Respectively, δ in ppm, J in Hz, in CD_3OD)

	1			**1a**
	C	H		C
1	134.1			135.5 (−1.4)[a]
2	127.5	5.61	ddq (5, 1, 1)	126.2
3	28.3	2.10eq	ddd (16, 5, 5)	28.1
		1.76ax	ddd (16, 13, 1)	
4	39.8	1.86	dddd (13,13, 5, 3)	39.9
5	29.9	2.20eq	ddd (14, 3, 2)	34.3 (−4.4)[a]
		1.24ax	ddd (14, 13, 3)	
6	76.1	4.13	dd (3, 2)	69.4 (+6.7) [a]
7	21.1	1.77	d (1)	21.2
8	73.0			72.8
9	27.8	1.18	s	27.2
10	26.1	1.14	s	27.0
1′	102.2	4.35	d (8)	
2′	75.2	3.17	dd (9, 8)	
3′	78.3	3.37	dd (9, 9)	
4′	72.0	3.278	m	
5′	78.0	3.284	m	
6′	63.1	3.88	dd (12, 2)	
		3.68	dd (12, 6)	

a) $\Delta\delta_{\mathbf{1}-\mathbf{1a}}$. m: Multiplet or overlapped signals.

carbon signals (three methyls, two methylenes, three methines, of which one is oxygenated, and two quaternary carbons) and the degree of unsaturation indicated the presence of a menthane skeleton as an aglycone. The doublet methyl signal at δ_H 1.77 (3H, d, J=1 Hz) must be long-range coupled with an olefinic proton, δ_H 5.61 (1H, ddq, J=5, 1, 1 Hz). The oxygenated quaternary carbon (δ_C 73.0) and two singlet methyl signals (δ_H 1.14, 1.18) were ascribable to an oxygenated isopropyl moiety. The double quantum-filtered (DQF) ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum revealed a proton coupling framework from H-2 to H-6, and the heteronuclear multiple bond correlation (HMBC) spectrum established the connectivity of the glucose at C-6 (Fig. 2a). The relative configuration of the aglycone was determined by considering the coupling constants and the phase-sensitive nuclear Overhauser effect spectroscopy (NOESY) spectral data. The axial coupling of H-4 [δ_H 1.86 (1H, dddd, J=13, 13, 5, 3 Hz)], the equatorial coupling of H-6 [δ_H 4.13 (1H, dd, J=3, 2 Hz)], and the NOE correlations around the six-membered ring portion were in good agreement with the relative configuration shown in Fig. 2b. In association with the absolute configuration of glucose, which was determined to be of the D-series by HPLC analysis following enzymatic hydrolysis of **1**, the 6*R* configuration was expected on application of the β-D-glucosylation-induced shift-trend rule.[7] In addition, this expectation was confirmed by the modified Mosher's method (Fig. 2c).[8] Therefore, myrseguinoside A (**1**) was elucidated to be (+)-*trans*-sobrerol 6-*O*-β-D-glucopyranoside.

Myrseguinoside B (**2**) was obtained as a yellow amorphous powder and its molecular formula was determined to be $C_{21}H_{20}O_{12}$ by HR-ESI-MS (m/z=487.0843 $[M+Na]^+$). The 12 degrees of unsaturation, the characteristic UV absorption (λ_{max} 252, 358 nm), and a chelated hydroxy group at δ_H 12.60 (1H, br s) indicated a flavonoid skeleton having 5-OH. The aromatic region of the ^{1}H-NMR spectrum of **2** contained four resonances exhibiting typical *meta*-coupling

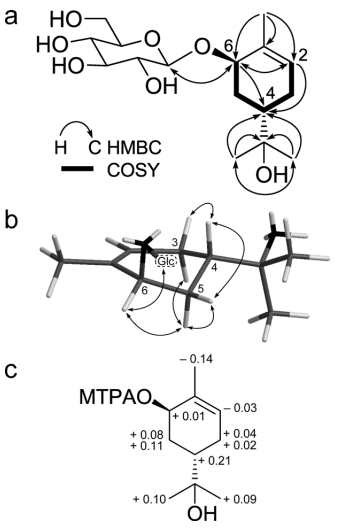

Fig. 2. Absolute Structure of **1**

(a) COSY (bold line) and HMBC (arrows) correlations. (b) Important NOESY correlations. (c) Analysis by the modified Mosher's method. The values are expressed as $\Delta\delta_{S-R}$.

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data for **2** and **3** (600, 150 MHz, Respectively, δ in ppm, J in Hz, in DMSO-d_6)

	2		**3**	
	C	H	C	H
2	156.22[a]	—	156.0	—
3	133.6	—	133.6	—
4	177.3	—	177.3	—
5	161.1	—	161.1	—
6	98.7	6.21 d (2)	98.9	6.19 d (2)
7	164.3	—	164.9	—
8	93.5	6.42 d (2)	93.9	6.47 d (2)
9	156.20[a]	—	156.4	—
10	103.8	—	103.7	—
1′	119.7	—	119.7	—
2′	109.2	7.14 d (2)	106.9	7.50 s
3′	145.3	—	147.5	—
4′	137.5	—	138.8	—
5′	147.7	—	147.5	—
6′	105.7	7.55 d (2)	106.9	7.50 s
OMe	56.1	3.85 s, 3H	56.3	3.86 s, 6H
1″	101.6	5.30 d (6)	101.7	5.33 d (6)
2″	70.9	3.74 br dd (7, 7)	71.0	3.72 dd (8, 6)
3″	71.8	3.50 dd (8, 3)	71.9	3.50 dd (8, 3)
4″	66.5	3.66 m	66.7	3.67 m
5″	64.9	3.29 m	65.2	3.30 dd (11, 1)
		3.65 dd (12, 4)		3.65 dd (11, 4)
5-OH		12.60 br s		12.61 br s

a) Interchangeable. m: Multiplet or overlapped signals.

(*J*=2 Hz), and the three oxygenated aromatic carbon signals (δ_C 147.7, 145.3, 137.5) on the B-ring were in accordance with a myricetin-related structure (Table 2).[1] The appearance of six signals for the B-ring suggested asymmetric substitution of it, *i.e.* a methoxy functionality (δ_C 56.1) on C-5′. The anomeric proton signal at δ_H 5.30 (1H, d, J=6 Hz) and five oxygenated carbon signals (δ_C 101.6, 71.8, 70.9, 66.5, 64.9) were attributable to an arabinopyranosyl group. Subsequent 2D-NMR [DQF-COSY, heteronuclear multiple quantum coherence (HMQC) and HMBC] analyses clarified the linkage of substitutions to be as shown in Fig. 3. Acid hydrolysis of **2**

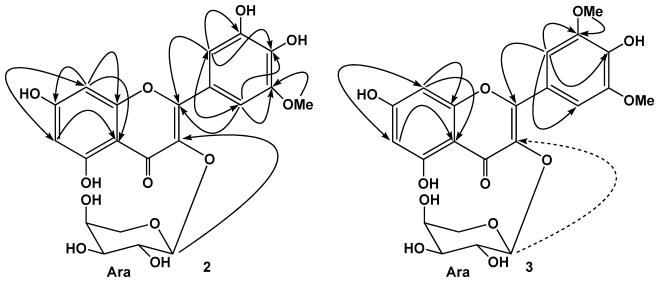

Fig. 3. HMBC Correlations for **2** and **3**
The dotted line indicates HMBC ($^3J_{CH}$=4 Hz).

followed by HPLC analysis with an optical rotation detector confirmed the L-configuration for arabinose. Thus, the structure of **2** was elucidated to be 5′-*O*-methylmyricetin 3-*O*-α-L-arabinopyranoside.

Myrseguinoside C (**3**) was obtained as a yellow amorphous powder and its molecular formula was determined to be $C_{22}H_{22}O_{12}$ by HR-ESI-TOF-MS (m/z=501.1006 $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectra were very similar to those of **2** except for the appearance of symmetric signals for the B-ring, *i.e.* an aromatic proton at δ_H 7.50 (2H, s) and methoxy protons at δ_H 3.86 (6H, s). These results coincided with the fact that the molecular formula of **3** was 14 mass units larger than that of **2**. Thus, compound **3** was expected to be the 3′-*O*-methyl ether of **2**. Full assignment of ^{1}H- and ^{13}C-NMR signals was achieved on the basis of the DQF-COSY, HMQC and HMBC spectra (Fig. 3). In the case of **3**, a HMBC pulse sequence optimized for $^3J_{CH}$ of 4 Hz was necessary to determine the sugar linkage, in which the anomeric proton (δ_H 5.33) was clearly correlated to C-3 (δ_C 133.6). Thus, myrseguinoside C (**3**) was determined to be 3′,5′-*O*-dimethylmyricetin 3-*O*-α-L-arabinopyranoside.

Myrseguinoside D (**4**) was obtained as a colorless amorphous powder. Its molecular formula, $C_{53}H_{84}O_{23}$, was determined from the molecular ion observed on HR-ESI-TOF-MS (m/z=1111.5285 $[M+Na]^+$). The IR spectrum showed absorption for hydroxyl and ester carbonyl functionalities (3395, 1753 cm^{-1}, respectively). The anomeric proton signals (δ_H 6.37, 5.36, 5.22, 4.92 ppm) and 23 oxygenated carbon signals suggested the presence of three hexoses and one pentose as sugar moieties, of which a rhamnopyranose and a glucopyranose were ascribable as terminal sugars due to the absence of any glycosylation shifts for these sugars as to ^{13}C-NMR resonances (Table 3). The remaining hexose was expected to be a glucopyranose exhibiting C-2 glycosylation due to the downfield resonance at δ_C 77.5. HPLC analysis of chiral derivatives of the acid hydrolysate of **4** revealed the presence of D-glucose, L-arabinose and L-rhamnose. The remaining 30 carbon signals were assignable to an olean-12-ene skeleton as an aglycone based on the six singlet methyl protons at δ_H 1.71, 1.29, 1.16, 1.02, 0.88 and 0.84 ppm, together with the typical olefinic carbon signals at δ_C 141.2 and 124.7. An ester carbonyl (δ_C 181.9) was expected to form a γ-lactone ring with one of the hydroxy groups considering the degree of unsaturation, the presence of an esterified proton signal [δ_H 5.43 (1H, dd, J=6, 3 Hz)], and a typical IR absorption at 1753 cm^{-1}. The structure was then fully elucidated by means of DQF-COSY, HMQC and HMBC spectra. The singlet methyl (δ_H 1.29, H-29) was correlated with C-19,

Table 3. ^{1}H- and ^{13}C-NMR Spectral Data for **4** and **5** (600, 150 MHz, Respectively, δ in ppm, J in Hz, in Pyridine-d_5)

	4			**5**		
	C	H		C	H	
1	38.9	0.87ax	m	39.3	0.91ax	m
		1.50eq	m		1.71eq	m
2	26.4	1.80ax	m	26.6	1.85ax	m
		2.00eq	m		2.05eq	m
3	89.1	3.17	dd (12, 5)	89.2	3.18	dd (12, 5)
4	39.5	—		39.7	—	
5	55.8	0.71	br d (12)	55.7	0.69	dd (11, 2)
6	18.5	1.26ax	m	18.0	1.38	m
		1.45eq	m		1.38	m
7	33.4	1.55ax	br dd (14, 13)	34.5	1.56ax	m
		1.31eq	m		1.23eq	m
8	40.0	—		42.5	—	
9	47.0	1.65	dd (11, 6)	50.5	1.30	dd (13, 2)
10	36.9	—		37.0	—	
11	23.7	1.78ax	m	19.3	1.82ax	m
		1.87eq	m		1.49eq	m
12	124.7	5.29	m	33.1	2.18	ddd (14, 14, 5)
					1.56	m
13	141.2	—		86.7	—	
14	42.51	—		44.7	—	
15	34.9	2.37ax	dd (16, 5)	37.0	2.28ax	dd (14, 5)
		1.71eq	m		1.52eq	dd (14, 1)
16	67.9	5.08	m	77.1	4.27	m
17	42.48	—		44.5	—	
18	42.52	2.23	dd (13, 7)	50.9	1.83	m
19	43.7	2.43ax	dd (13, 13)	34.3	2.73ax	dd (15, 13)
		1.79eq	m		2.10eq	m
20	43.9	—		41.0	—	
21	40.9	3.74ax	dd (12, 3)	32.4	2.53ax	ddd (13, 13, 6)
		2.16eq	dd (12, 6)		1.97eq	m
22	80.4	5.43	dd (6, 3)	31.7	1.75ax	ddd (13, 13, 6)
					2.00eq	m
23	28.1	1.16	s	28.1	1.17	s
24	16.7	1.02	s	16.54	1.04	s
25	15.7	0.84	s	16.46	0.88	s
26	17.3	0.88	s	18.6	1.36	s
27	27.5	1.71	s	19.6	1.56	s
28	65.3	3.67	d (11)	77.9	3.42	d (7)
		4.05	m		3.66	d (7)
29	21.0	1.29	s	24.1	1.26	s
30	181.9	—		108.9	4.68	s
OMe	—	—		58.6	3.56	s
OMe	—	—		58.4	3.54	s
Ara						
1′	104.4	4.92	m	104.4	4.95	m
2′	80.6	4.56	dd (7, 5)	80.7	4.56	dd (7, 5)
3′	72.4	4.46	m	72.3	4.49	m
4′	74.77[a)]	4.57	m	74.8	4.57	m
5′	63.5	3.76	m	63.7	3.81	m
		4.40	dd (12, 6)		4.40	m
Glc						
1″	103.1	5.22	d (8)	103.2	5.22	d (8)
2″	77.5	4.26	br t (9)	77.5	4.26	dd (9, 8)
3″	79.5	4.18	dd (9, 9)	79.5	4.18	dd (9, 9)
4″	71.9	4.11	dd (9, 9)	71.95	4.11	dd (9, 9)
5″	78.3	3.80	m	78.3	3.80	ddd (9, 5, 2)
6″	62.7	4.26	m	62.7	4.26	m
		4.42	dd (12, 2)		4.44	dd (12, 2)
Rha						
1‴	101.6	6.37	br s	101.6	6.37	d (1)
2‴	72.4	4.71	dd (3, 1)	72.4	4.71	dd (3, 1)
3‴	72.7	4.66	dd (9, 3)	72.7	4.66	dd (10, 3)
4‴	74.80[a)]	4.27	m	74.8	4.27	m
5‴	69.5	4.99	m	69.5	5.01	dq (10, 6)
6‴	18.9	1.80	d (6)	18.9	1.80	d (6)
Glc						
1⁗	105.4	5.36	d (8)	105.4	5.35	d (8)
2⁗	76.4	4.05	br t (9)	76.4	4.06	dd (8, 9)
3⁗	78.1	4.29	dd (9, 9)	78.08[a)]	4.29	dd (9, 9)
4⁗	71.8	4.21	dd (9, 9)	71.85	4.21	dd (9, 9)
5⁗	78.1	4.05	m	78.05[a)]	4.05	m
6⁗	62.9	4.36	dd (12, 5)	62.9	4.36	dd (12, 5)
		4.48	dd (12, 2)		4.49	m

a) Interchangeable in each column. m: Multiplet or overlapped signals.

20, 21 and 30, the esterified methine proton (δ_H 5.43, H-22) with C-18, 28 and 30, and the carbinol protons (δ_H 3.67, 4.05, H-28) with C-16 and 22, which revealed the structure of an aglycone, as shown in Fig. 4. The sugar linkage was established from the HMBC correlations between H-1‴ (δ_H 6.37, Rha) and C-2″ (δ_C 77.5, inner Glc), between H-1⁗ (δ_H 5.36, terminal Glc) and C-2′ (δ_C 80.6, Ara), between H-1″ (δ_H 5.22, inner Glc) and C-4′ (δ_C 74.77, Ara), and between H-1′ (δ_H 4.92, Ara) and C-3 (δ_C 89.1, aglycone) (Fig. 4). The structure of the sugar chain was finally confirmed by comparing the ^{1}H- and ^{13}C-NMR chemical shift values with those of related known compounds having the same sugar residue, *i.e.* ardisiacrispin B (**12**) and ardisicrenoside A (**13**).[9] Several researchers have reported the rapid conformational equilibrium between the $^1C_4$ and $^4C_1$ conformers of α-L-arabinopyranosides to reduce the steric hindrance caused by the C-2 substitution.[10,11] The signal broadening for the arabinose moiety in the ^{1}H- and ^{13}C-NMR spectra of **4** was also observed in our experiments.

The axial–axial coupling between H-18 [δ_H 2.23 (1H, dd, J=13, 7 Hz)] and H-19ax [δ_H 2.43 (1H, dd, J=13, 13 Hz)], and the equatorial–axial and equatorial–equatorial coupling to H-22 [δ_H 5.43 (1H, dd, J=6, 3 Hz)] suggested the stereochemistry around the E-ring to be as shown in Fig. 1. The NOE correlations between H-18 and H-19β (δ_H 1.79), and between H-19ax (δ_H 2.43) and H-21α (δ_H 3.74) also confirmed the stereochemistry of **4** (Fig. 5). Thus, based on this evidence, the structure of **4** was established to be 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-16α,22β,28-trihydroxyolean-12-en-30,22β-olide.

Myrseguinoside E (**5**) was obtained as an colorless amorphous powder and its molecular formula was determined to be $C_{55}H_{92}O_{23}$ from the molecular ion observed on HR-ESI-MS (m/z=1143.5912 $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR resonances were very similar to those of **12**. The presence of an acetal carbon (δ_C 108.9) and two methoxy signals (δ_C 58.6, 58.4) together with its molecular formula suggested the structure of **5** was that of a dimethyl acetal of **12**. This was confirmed by DQF-COSY, HMQC, HMBC and phase-sensitive ROESY analyses. Tommasi *et al.* reported a similar triterpene saponin having a dimethyl acetal as a new compound from the same family, *Ardisia japonica*.[12] However, **5** could be an artefact due to the use of MeOH during the purification process.

Compounds **1**—**5** were examined for their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities, and also tumor cell growth inhibitory activities toward HL-60 and SBC-3 by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Table 4). While myrseguinoside B (**2**) (IC_{50}=30.9±3.20 μM) showed approximately half the activity of the reference compound, Trolox (IC_{50}=16.2±0.29 μM), the methyl derivative of **2**, *i.e.* myrseguinoside C (**3**), did not exhibit significant activity at 50 μM. On the basis of this observation, the *ortho*-dihydroxy groups in **2** were judged to be important for the radical-scavenging activity of **2**. Myrseguinoside E (**5**) exhibited moderate growth inhibitory activity toward HL-60 (IC_{50}=23.7±1.61 μM) and SBC-3 (IC_{50}=10.9±2.00 μM). Chang *et al.* reported the cytotoxic activity of ardisiacrispin B (**12**) toward

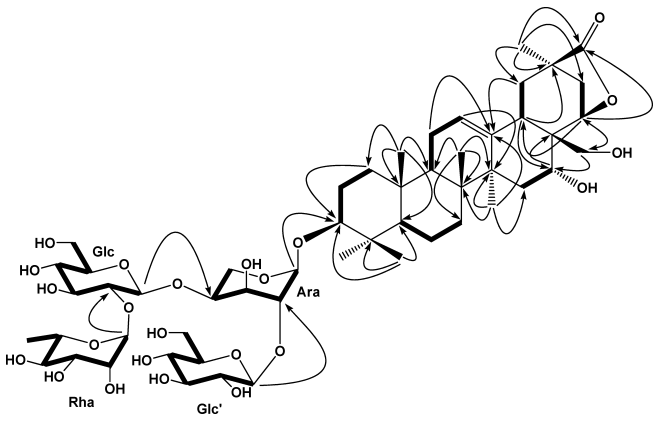

Fig. 4. COSY (Bold Lines) and HMBC (Arrows) Correlations for **4**

Table 4. DPPH Radical-Scavenging and Tumor Cell Growth Inhibitory Activities of **1**—**5**

	DPPH	HL-60	SBC-3
1	NA	NA	NA
2	78.1±4.77 (30.9±3.20)	NA	NA
3	NA	NA	NA
4	NA	NA	34.2±6.89
5	NA	99.1±1.46 (23.7±1.61)	100.1±0.24 (10.9±2.00)
Trolox (20 μM)	55.7±8.37 (16.2±0.29)	—	—
Etoposide (1 μM)	—	34.5±9.61 (1.28±0.14)	66.3±3.93 (0.34±0.17)

NA: Not active. The upper and lower values indicate % inhibition at 50 μM and IC_{50} (μM), respectively.

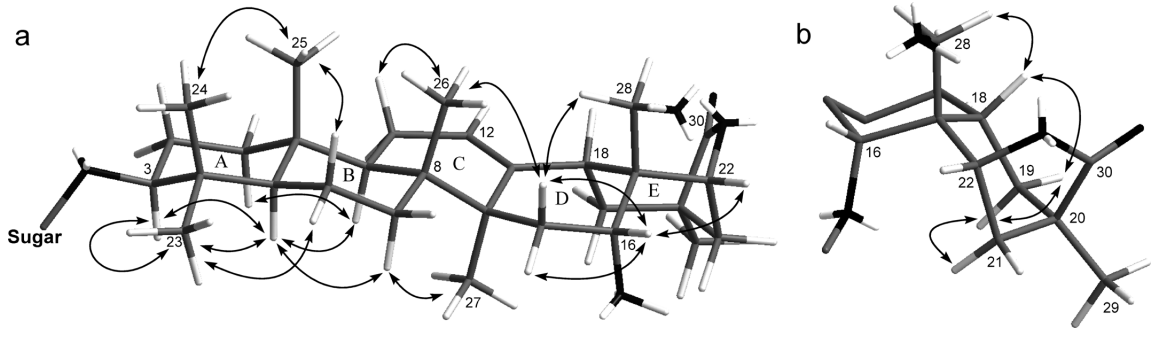

Fig. 5. Important NOESY Correlations

(a) Front view of **4**. (b) Side view of the D, E and F rings.

HL-60 on IC_{50} value of $3.2 \pm 1.61\,\mu$M, while ardisicrenoside A (**13**) was inactive ($>100\,\mu$M).[13] Considerating these results together with the weak activity of **4**, the substituents around the E-ring may be important for tumor cell growth inhibitory activity.

Experimental

General Experimental Procedures Silica gel column chromatography (CC) was performed on silica gel 60 (Merck, Darmstadt, Germany), and reversed-phase [octadecyl silica gel (ODS)] open CC (RPCC) on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) (Φ=4 cm, L=20 cm). HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ=10 mm, L=250 mm), and the eluate was monitored with a refractive index monitor.

Optical rotations were measured on a JASCO P-1030 polarimeter. IR spectra were measured on a Horiba FT-710 Fourier transform infrared spectrophotometer and UV spectra on a JASCO V-520 UV/Vis spectrophotometer. NMR spectra were taken on a JEOL JNM-ECA 600 spectrometer at 600 MHz for ^{1}H, and 150 MHz for ^{13}C, respectively, with tetramethylsilane (TMS) as an internal standard. Positive-ion HR-ESI-TOF-MS was recorded on an Applied Biosystem QSTAR XL spectrometer. VERSA Max (Molecular Device, Silicon Valley, CA, U.S.A.) was used as a microplate reader.

Plant Material Fruits of *M. seguinii* were collected in Okinawa, Japan, in July 1992, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University (No. 92-MS-Okinawa-0727).

Extraction and Isolation The air-dried fruits of *M. seguinii* (670 g) were extracted with MeOH (4 l) three times by maceration. The MeOH extracts were combined and evaporated to dryness under vacuum to afford a viscous gummy material (55.0 g). This residue (55.0 g) was applied to a silica gel column (Φ=5 cm, L=50 cm) using a stepwise gradient containing increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (3 l), $CHCl_3$–MeOH (10 : 1, 3 l), (5 : 1, 3 l), (3 : 1, 3 l), and (2 : 1, 3 l), and MeOH (3 l)]. The residue (2.12 g) of the $CHCl_3$–MeOH (5 : 1) eluate obtained on silica gel CC was subsequently subjected to RPCC with stepwise gradient elution with increasing amounts of MeOH in H_2O (10, 30, 50, 70, 90, 100% MeOH, 1 l). The residue (300 mg) obtained from the 50% MeOH eluate was further applied to a Sephadex LH-20 column (Φ=2 cm, L=120 cm) with MeOH as an eluate, 5-ml fractions (frs) being collected. The residue (264 mg in frs 1—38) was further separated by HPLC (ODS) with 20% CH_3CN to afford **1** (9.2 mg) from the peak at 9 min (flow rate: 3.0 ml/min). The residue (19.4 mg in frs 39—58) was further separated by HPLC (ODS) with 20% CH_3CN to afford **9** (3.2 mg) and **8** (2.0 mg) from the peaks at 17 and 27 min, respectively (flow rate: 3.0 ml/min). The residue (24.2 mg in frs 59—74) was further separated by HPLC (ODS) with 25% CH_3CN to afford **3** (12.8 mg) from the peak at 12 min (flow rate: 3.0 ml/min).

The residue (2.57 g) of the $CHCl_3$–MeOH (3 : 1) eluate obtained on silica gel CC was subsequently subjected to RPCC with stepwise gradient elution with increasing amounts of MeOH in H_2O (10, 30, 50, 70, 90, 100% MeOH, 1 l). The residue (1.00 g) obtained from the 10% MeOH eluate was further applied to a Sephadex LH-20 column (Φ=2 cm, L=120 cm) with MeOH as an eluate, 5-ml frs being collected. The residue (510 mg in frs 25—45) was further separated by HPLC (ODS) with 3% CH_3CN to afford **10** (6.2 mg) and **11** (6.8 mg) from the peaks at 11 min and 25 min, respectively (flow rate: 2.8 ml/min).

The residue (7.26 g) of the $CHCl_3$–MeOH (2 : 1) eluate obtained on silica gel CC was subsequently subjected to RPCC with stepwisw gradient elution with increasing amounts of MeOH in H_2O (10, 30, 50, 70, 90, 100% MeOH, 1 l). The residue (218 mg) obtained from the 50% MeOH eluate was further applied to a Sephadex LH-20 column (Φ=2 cm, L=120 cm) with MeOH as an eluate, 5-ml frs being collected. The residue (46.7 mg in frs 34—57) was further separated by HPLC (ODS) with 25% CH_3CN to afford **7** (8.5 mg), **2** (2.4 mg), and **6** (3.3 mg) from the peaks at 11, 14, and 17 min, respectively (flow rate: 3.0 ml/min). The residue (217 mg) obtained from the 70% MeOH eluate was further applied to a Sephadex LH-20 column (Φ=2 cm, L=120 cm) with MeOH as an eluate, 5 ml fractions (frs) being collected. The residue (102.2 mg in frs 9—19) was further separated by HPLC (ODS) with 35% CH_3CN to afford **4** (8.1 mg), **13** (16.9 mg), **12** (15.6 mg), and **5** (7.8 mg) from the peaks at 7, 9, 21, and 28 min, respectively (flow rate: 3.0 ml/min).

The known compounds (**6**—**13**) were identified by comparison of the spectroscopic data with those reported in the literature, as follows. Quercitrin (**6**), $[\alpha]_D^{27}$ −100.2 (c=0.22, MeOH),[1] myricitrin (**7**), $[\alpha]_D^{27}$ −109.6 (c=0.55, MeOH),[1] kaempferol 3-*O*-glucoside (**8**), $[\alpha]_D^{23}$ −13.9 (c=0.11, MeOH),[14] syringetin 3-*O*-glucoside (**9**), $[\alpha]_D^{25}$ −11.9 (c=0.16, MeOH),[15] arbutin (**10**), $[\alpha]_D^{24}$ −45.6 (c=0.62, MeOH),[3] seguinoside A (**11**), $[\alpha]_D^{25}$ −72.9 (c=0.68, MeOH),[3] ardisiacrispin B (**12**), $[\alpha]_D^{27}$ −16.7 (c=1.56, MeOH),[9] and ardisicrenoside A (**13**), $[\alpha]_D^{26}$ −17.2 (c=1.69, MeOH).[9]

Myrseguinoside A (**1**): Amorphous powder; $[\alpha]_D^{27}$ +20.1 (c=0.80, MeOH); IR ν_{max} (film) cm^{-1}: 3382, 2969, 2919, 1604, 1550, 1382, 1278, 1077, 1031, 920; ^{1}H- and ^{13}C-NMR (CD_3OD): Table 1; HR-ESI-TOF-MS (positive-ion mode) m/z: 355.1722 $[M+Na]^+$ (Calcd for $C_{16}H_{28}O_7Na$: 355.1727).

Myrseguinoside B (**2**): Amorphous powder; $[\alpha]_D^{26}$ −38.6 (c=0.08, MeOH); IR ν_{max} (film) cm^{-1}: 3393, 2961, 2851, 1650, 1603, 1506, 1361, 1201, 1087, 1025; UV λ_{max} (MeOH) nm (log ε): 358 (4.00), 252 (4.08), 211 (4.35); ^{1}H- and ^{13}C-NMR (DMSO-d_6): Table 2; ^{1}H-NMR (CD_3OD) δ_H: 7.54 (1H, d, J=2 Hz, H-6′), 7.31 (1H, d, J=2 Hz, H-2′), 6.41 (1H, d, J=2 Hz, H-8), 6.21 (1H, d, J=2 Hz, H-6), 5.22 (1H, d, J=7 Hz, H-1″), 3.94 (3H, s, OMe), 3.90 (1H, dd, J=8, 7 Hz, H-2″), 3.83 (1H, dd, J=12, 3 Hz, H-5″a), 3.81 (1H, ddd, J=3, 3, 1 Hz, H-4″), 3.64 (1H, dd, J=8, 3 Hz, H-3″), 3.45 (1H, dd, J=12, 1 Hz, H-5″b); ^{13}C-NMR (CD_3OD) δ_C: 179.6 (C-4), 166.2 (C-7), 163.2 (C-5), 158.7 (C-9), 158.5 (C-2), 149.3 (C-5′), 146.4 (C-3′), 139.0 (C-4′), 135.8 (C-3), 121.9 (C-1′), 111.1 (C-2′), 106.9 (C-6′), 105.8 (C-10), 104.5 (C-1″), 100.0 (C-6), 94.8 (C-8), 74.2 (C-3″), 73.0 (C-2″), 69.2 (C-4″), 67.2 (C-5″), 57.2 (OMe); HR-ESI-TOF-MS (positive-ion mode) m/z: 487.0843 $[M+Na]^+$ (Calcd for $C_{21}H_{20}O_{12}Na$: 487.0846).

Myrseguinoside C (**3**): Amorphous powder; $[\alpha]_D^{27}$ −23.3 (c=0.18, MeOH); IR ν_{max} (film) cm^{-1}: 3363, 2959, 2849, 1652, 1607, 1508, 1359, 1198, 1081, 1021; UV λ_{max} (MeOH) nm (log ε): 360 (3.84), 251 (3.88), 210 (4.17); ^{1}H- and ^{13}C-NMR (DMSO-d_6): Table 2; ^{1}H-NMR (CD_3OD) δ_H: 7.55 (2H, s, H-2′, 6′), 6.37 (1H, d, J=2 Hz, H-8), 6.17 (1H, d, J=2 Hz, H-6), 5.21 (1H, d, J=7 Hz, H-1″), 3.94 (6H, s, OMe), 3.89 (1H, dd, J=9, 7 Hz, H-2″), 3.82 (1H, dd, J=12, 3 Hz, H-5″a), 3.80 (1H, ddd, J=3, 3, 1 Hz, H-4″), 3.64 (1H, dd, J=9, 3 Hz, H-3″), 3.45 (1H, dd, J=12, 1 Hz, H-5″b); ^{13}C-NMR (CD_3OD) δ_C: 179.7 (C-4), 166.9 (C-7), 163.0 (C-5), 158.7 (C-9), 158.2 (C-2), 149.1 (C-3′, 5′), 140.2 (C-4′), 135.7 (C-3), 122.0 (C-1′), 108.3 (C-2′, 6′), 105.1 (C-10), 104.6 (C-1″), 100.9 (C-6), 95.5 (C-8), 74.3 (C-3″), 73.1 (C-2″), 69.3 (C-4″), 67.3 (C-5″), 57.3 (OMe); HR-ESI-TOF-MS (positive-ion mode) m/z: 501.1006 $[M+Na]^+$ (Calcd for $C_{22}H_{22}O_{12}Na$: 501.1003).

Myrseguinoside D (**4**): Amorphous powder; $[\alpha]_D^{25}$ −12.9 (c=0.81, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2928, 1753, 1649, 1511, 1457, 1261, 1073, 1039; ^{1}H- and ^{13}C-NMR (C_5D_5N): Table 3; ^{1}H-NMR (CD_3OD) δ_H: 5.32 (1H, dd, J=3, 3 Hz, H-12), 5.28 (1H, d, J=2 Hz, H-1‴), 4.87 (1H, br d, J=6 Hz, H-22), 4.62 (1H, d, J=8 Hz, H-1″), 4.60 (1H, d, J=8 Hz, H-1⁗), 4.51 (1H, d, J=5 Hz, H-1′), 4.29 (1H, dd, J=4, 4 Hz, H-16), 4.11 (1H, dq, J=9, 6 Hz, H-5‴), 4.05 (1H, m, H-4′), 4.05 (1H, dd, J=13, 5 Hz, H-5′a), 3.93 (1H, dd, J=3, 2 Hz, H-2‴), 3.87 (1H, m, H-2′), 3.86 (1H, m, H-3′), 3.85 (1H, m, H-6⁗a), 3.82 (1H, dd, J=12, 2 Hz, H-6″a), 3.75 (1H, dd, J=10, 3 Hz, H-3‴), 3.66 (2H, m, H-6″b, H-6⁗b), 3.55 (1H, m, H-5′b), 3.44 (1H, m, H-2″), 3.43 (1H, m, H-3″), 3.42 (1H, d, J=12 Hz, H-28a), 3.39 (1H, dd, J=9, 9 Hz, H-3⁗), 3.38 (1H, dd, J=10, 9 Hz, H-4‴), 3.28 (1H, m, H-4″, H-5⁗), 3.27 (1H, m, H-4⁗), 3.25 (1H, m, H-5″), 3.22 (1H, d, J=12 Hz, H-28b), 3.19 (1H, br d, J=11 Hz, H-21ax), 3.19 (1H, dd, J=9, 8 Hz, H-2⁗), 3.15 (1H, dd, J=12, 5 Hz, H-3), 2.15 (1H, dd, J=13, 13 Hz, H-19ax), 2.09 (1H, m, H-15ax), 2.05 (1H, ddd, J=11, 6, 2 Hz, H-21 eq), 1.90 (2H, m, H_2-11), 1.86 (1H, m, H-2 eq), 1.78 (1H, dd, J=13, 6 Hz, H-18), 1.74 (1H, m, H-2ax), 1.73 (1H, m, H-1eq), 1.65 (1H, m, H-9), 1.61 (1H, m, H-7ax), 1.60 (1H, m, H-6eq), 1.51 (1H, ddd, J=13, 6, 2 Hz, H-19eq), 1.46 (1H, m, H-7eq), 1.45 (1H, m, H-6ax), 1.44 (1H, m, H-15eq), 1.40 (3H, s, H-27), 1.30 (1H, d, J=6 Hz, H-6‴), 1.13 (3H, s, H-29), 1.06 (3H, s, H-23), 0.98 (1H, m, H-1ax), 0.98 (3H, s, H-25), 0.94 (3H, s, H-26), 0.85 (3H, s, H-24), 0.80 (1H, br d, J=12 Hz, H-5); ^{13}C-NMR (CD_3OD) δ_C: 184.3 (C-30), 141.7 (C-13), 125.9 (C-12), 105.2 (C-1′), 104.7 (C-1″), 104.0 (C-1⁗), 101.9 (C-1‴), 90.9 (C-3), 81.4 (C-22), 79.7 (C-2′), 79.1 (C-2″), 78.9 (C-3″), 78.0 (C-3⁗), 77.9 (C-5⁗), 77.7 (C-5″), 76.6 (C-4′), 76.4 (C-2⁗), 74.5 (C-4⁗), 72.8 (C-3′), 72.3 (C-2‴), 72.1 (C-3‴), 71.9 (C-4‴), 71.7 (C-4″), 70.4 (C-5‴), 68.9 (C-16), 66.1 (C-28), 64.7 (C-5′), 62.9 (C-6⁗), 62.8 (C-6″), 57.1 (C-5), 48.2 (C-9), 44.3 (C-20), 44.2 (C-19), 43.6 (C-18), 43.5 (C-14), 43.3 (C-17), 41.5 (C-21), 41.1 (C-8), 40.4 (C-4), 40.0 (C-1), 37.9 (C-10), 35.6 (C-15), 34.3 (C-7), 28.7 (C-23), 27.9 (C-27), 27.1 (C-2), 24.5 (C-11), 20.9 (C-29), 19.4 (C-6), 18.3 (C-6‴), 18.1 (C-26), 17.0 (C-24), 16.3 (C-25); HR-ESI-TOF-MS (positive-ion mode) m/z: 1111.5285 $[M+Na]^+$ (Calcd for $C_{53}H_{84}O_{23}Na$: 1111.5295).

Myrseguinoside E (**5**): Amorphous powder; $[\alpha]_D^{26}$ −14.2 (c=0.50, MeOH); IR ν_{max} (film) cm^{-1}: 3399, 2927, 1649, 1509, 1455, 1260, 1073, 1044; ^{1}H- and ^{13}C-NMR (C_5D_5N): Table 3; ^{1}H-NMR (CD_3OD) δ_H: 5.28 (1H, d, J=2 Hz, H-1‴), 4.61 (1H, d, J=8 Hz, H-1″), 4.60 (1H, d, J=8 Hz, H-

1‴″), 4.51 (1H, d, J=5 Hz, H-1′), 4.11 (1H, dq, J=10, 6 Hz, H-5‴), 4.05 (1H, dd, J=12, 5 Hz, H-5′a), 4.04 (1H, m, H-4′), 3.93 (1H, dd, J=3, 2 Hz, H-2‴), 3.91 (1H, br d, J=5 Hz, H-16), 3.86 (1H, dd, J=12, 2 Hz, H-6‴″a), 3.86 (1H, m, H-3′), 3.86 (1H, m, H-2′), 3.75 (1H, dd, J=10, 3 Hz, H-3‴), 3.82 (1H, dd, J=12, 2 Hz, H-6″a), 3.66 (1H, dd, J=12, 5 Hz, H-6‴″b), 3.65 (1H, dd, J=12, 5 Hz, H-6″b), 3.55 (1H, m, H-5′b), 3.51 (1H, m, H-28a), 3.44 (1H, m, H-2″), 3.44 (1H, m, H-3″), 3.39 (1H, dd, J=9, 9 Hz, H-3‴″), 3.38 (1H, dd, J=10, 10 Hz, H-4‴), 3.28 (1H, m, H-4‴″, H-5‴″, H-4″), 3.25 (1H, ddd, J=10, 5, 2 Hz, H-5″), 3.19 (1H, dd, J=9, 8 Hz, H-2‴″), 3.16 (1H, d, J=8 Hz, H-28b), 3.13 (1H, dd, J=12, 4 Hz, H-3), 2.23 (1H, dd, J=14, 13 Hz, H-19ax), 2.12 (1H, dd, J=15, 6 Hz, H-15ax), 2.06 (1H, ddd, J=14, 14, 5 Hz, H-12ax), 1.96 (1H, ddd, J=14, 14, 5 Hz, H-21ax), 1.86 (1H, m, H-2eq), 1.79 (1H, m, H-22eq), 1.75 (1H, m, H-1eq), 1.74 (1H, m, H-2ax), 1.74 (1H, m, H-19eq), 1.62 (1H, br dd, J=14, 4 Hz, H-21eq), 1.56 (1H, m, H-22ax), 1.54 (1H, m, H-7ax), 1.54 (1H, m, H-18), 1.52 (1H, m, H-11ax), 1.52 (1H, m, H-6eq), 1.47 (1H, m, H-11eq), 1.46 (1H, m, H-6ax), 1.30 (1H, d, J=6 Hz, H-6‴), 1.28 (1H, m, H-12eq), 1.26 (1H, m, H-9), 1.23 (3H, s, H-27), 1.23 (1H, m, H-7eq), 1.22 (1H, m, H-15eq), 1.16 (3H, s, H-26), 1.04 (3H, s, H-23), 0.97 (1H, m, H-1ax), 0.90 (3H, s, H-25), 0.85 (3H, s, H-29), 0.82 (3H, s, H-24), 0.73 (1H, dd, J=12, 2 Hz, H-5); ^{13}C-NMR (CD_3OD) δ_C: 110.4 (C-30), 105.2 (C-1′), 104.7 (C-1″), 103.9 (C-1‴″), 101.9 (C-1‴), 91.0 (C-3), 88.5 (C-13), 79.7 (C-2′), 79.1 (C-2″), 78.9 (C-3″), 78.6 (C-28), 77.99 (C-3‴″), 77.96 (C-5‴″), 77.94 (C-16), 77.7 (C-5″), 76.6 (C-4′), 76.4 (C-2‴″), 74.5 (C-4‴), 72.8 (C-3′), 72.3 (C-2‴), 72.1 (C-3‴), 71.9 (C-4‴″), 71.7 (C-4″), 70.4 (C-5‴), 64.7 (C-5′), 62.9 (C-6‴″), 62.8 (C-6″), 56.9 (C-5), 51.6 (C-18), 51.5 (C-9), 45.4 (C-14), 45.1 (C-17), 43.4 (C-8), 41.5 (C-20), 40.5 (C-4), 40.3 (C-1), 37.9 (C-10), 37.1 (C-15), 35.2 (C-7), 35.0 (C-19), 33.6 (C-12), 32.8 (C-21), 31.9 (C-22), 28.6 (C-23), 27.2 (C-2), 23.9 (C-29), 19.94 (C-27), 19.92 (C-11), 18.85 (C-26), 18.82 (C-6), 18.3 (C-6‴), 16.81 (C-24), 16.79 (C-25); HR-ESI-TOF-MS (positive-ion mode) m/z: 1143.5912 $[M+Na]^+$ (Calcd for $C_{55}H_{92}O_{23}Na$: 1143.5921).

The identification of **12** and **13** was performed by comparison of the ^{1}H- and ^{13}C-NMR data (in C_5D_5N) with those reported in the literature.[9] The ^{13}C-NMR data (in CD_3OD) are provided here because of their unavailability on other journals. Ardisiacrispin B (**12**): ^{13}C-NMR (CD_3OD) δ_C: 209.3 (C-30), 105.2 (C-1′), 104.7 (C-1″), 103.9 (C-1‴″), 101.9 (C-1‴), 91.0 (C-3), 88.2 (C-13), 79.7 (C-2′), 79.1 (C-2″), 78.9 (C-3″), 78.5 (C-28), 78.0 (C-3‴″), 77.9 (C-5‴″), 77.9 (C-16), 77.7 (C-5″), 76.5 (C-4′), 76.4 (C-2‴″), 74.5 (C-4‴), 72.7 (C-3′), 72.3 (C-2‴), 72.1 (C-3‴), 71.9 (C-4‴″), 71.7 (C-4″), 70.4 (C-5‴), 64.7 (C-5′), 62.9 (C-6‴″), 62.8 (C-6″), 56.9 (C-5), 54.0 (C-18), 51.4 (C-9), 45.4 (C-14), 44.8 (C-17), 43.5 (C-8), 49.0 (C-20, overlapped with CD_3OD), 40.5 (C-4), 40.3 (C-1), 37.9 (C-10), 37.1 (C-15), 35.2 (C-7), 34.1 (C-19), 33.3 (C-12), 32.8 (C-21), 31.0 (C-22), 28.6 (C-23), 27.2 (C-2), 24.3 (C-29), 20.2 (C-27), 19.8 (C-11), 18.85 (C-26), 18.82 (C-6), 18.3 (C-6‴), 16.81 (C-24), 16.75 (C-25). Ardisicrenoside A (**13**): ^{13}C-NMR (CD_3OD) δ_C: 105.2 (C-1′), 104.7 (C-1″), 103.9 (C-1‴″), 101.9 (C-1‴), 91.0 (C-3), 88.5 (C-13), 79.7 (C-2′), 79.1 (C-2″), 78.9 (C-3″), 78.7 (C-28), 78.0 (C-3‴″), 77.95 (C-5‴″), 78.1 (C-16), 77.7 (C-5″), 76.6 (C-4′), 76.4 (C-2‴″), 74.5 (C-4‴), 72.8 (C-3′), 72.3 (C-2‴), 72.1 (C-3‴), 71.9 (C-4‴″), 71.7 (C-4″), 70.4 (C-5‴), 66.8 (C-30), 64.7 (C-5′), 62.9 (C-6‴″), 62.8 (C-6″), 56.9 (C-5), 51.8 (C-18), 51.5 (C-9), 45.4 (C-14), 45.3 (C-17), 43.4 (C-8), 37.0 (C-20), 40.5 (C-4), 40.3 (C-1), 37.9 (C-10), 37.2 (C-15), 35.2 (C-7), 34.0 (C-19), 33.4 (C-12), 32.9 (C-21), 31.9 (C-22), 28.61 (C-23), 27.2 (C-2), 28.56 (C-29), 20.1 (C-27), 19.9 (C-11), 18.9 (C-26), 18.8 (C-6), 18.3 (C-6‴), 16.81 (C-24), 16.78 (C-25).

Enzymatic Hydrolysis of 1 Compound **1** (4.0 mg) was hydrolyzed with crude hesperidinase at 37 °C in 1 ml of H_2O with reciprocal shaking for 12 h. Liberation of glucose was monitored by TLC analysis ($CHCl_3$: MeOH : H_2O, 15 : 6 : 1, Rf values, **1**: 0.52, aglycone **1a**: 0.80, and glucose: 0.11). The reaction mixture was concentrated and then subjected to silica gel CC [8 g, Φ=1 cm, L=20 cm, linear gradient, $CHCl_3$–MeOH (10 : 1, 100 ml)→$CHCl_3$–MeOH–H_2O (15 : 6 : 1, 100 ml)→EtOH 50 ml, 10-g fractions being collected]. The aglycone, **1a** (0.58 mg), and glucose were recovered in fractions 11—12 and 41—42, respectively. The absolute configuration of glucose was determined to be of the D-series by the positive optical rotation sign and the retention time (13.7 min) on HPLC analysis [JASCO OR-2090 Plus; optical rotation detector, SHODEX Asahipak NH2P-50; Φ=4.5 mm, L=25 cm, 80% CH_3CN aq., 1 ml/min]. Peaks were identified by co-chromatography with authentic D-glucose. **1a**: Amorphous powder; $[\alpha]_D^{27}$ +192 (c=0.058, MeOH); IR ν_{max} (film) cm^{-1}: 3339, 2971, 2921, 1648, 1556, 1463, 1376, 1251, 1157, 859; ^{1}H-NMR (CD_3OD) δ_H: 5.56 (1H, ddq, J=5, 1, 1 Hz, H-2), 3.96 (1H, dd, J=4, 2 Hz, H-6), 2.10 (1H, m, H-3eq), 1.96 (1H, ddd, J=13, 4, 2 Hz, H-5eq), 1.79 (2H, m, H-3ax, H-4), 1.76 (3H, br d, J=1 Hz, H_3-7), 1.37 (1H, ddd, J=13, 12, 4 Hz, H-5ax), 1.16 (6H, s, H_3-9, H_3-10); ^{13}C-NMR (CD_3OD): Table 1; HR-ESI-TOF-MS (positive-ion mode) m/z: 193.1201 $[M+Na]^+$ (Calcd for $C_{10}H_{18}O_2Na$: 193.1199).

Preparation of (*R*)- and (*S*)-MTPA Esters (1b and 1c) of 1a A solution of **1a** (0.29 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) (20.1 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (18.7 mg) and 4-*N*,*N*′-dimethylaminopyridine (DMAP) (9.8 mg), followed by stirring at 25 °C for 30 min and then 40 °C for 5 min. After the addition of 1.0 ml each of H_2O and $CHCl_3$, the solution was washed with 1 M HCl (1 ml), $NaHCO_3$-saturated H_2O (1 ml), and saturated brine (1 ml) successively. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness, applied for 18 cm and developed with $CHCl_3$–$(CH_3)_2CO$ (20 : 1) for 9 cm and eluted with $CHCl_3$–MeOH (9 : 1))] to furnish a monoester, **1b** (0.24 mg). Through a similar procedure, **1c** (0.31 mg) was also prepared from **1a** (0.29 mg) using (*S*)-MTPA (18.6 mg), EDC (17.3 mg), and DMAP (10.4 mg). (*R*)-MTPA ester, (**1b**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ_H: 7.58—7.56 (2H, m, aromatic protons), 7.40—7.38 (3H, m, aromatic protons), 5.76 (1H, br d, J=5 Hz, H-2), 5.47 (1H, m, H-6), 3.58 (3H, br s, OMe), 2.13 (1H, br d, J=17 Hz, H-3 eq), 2.06 (1H, m, H-5eq), 1.81 (1H, m, H-3ax), 1.73 (3H, br s, H_3-7), 1.49 (1H, m, H-5ax), 1.48 (1H, m, H-4), 1.08 (6H, s, H_3-9, 10); HR-ESI-TOF-MS (positive-ion mode) m/z: 409.1594 $[M+Na]^+$ (Calcd for $C_{20}H_{25}O_4F_3Na$: 409.1597). (*S*)-MTPA ester, (**1c**): Amorphous powder; ^{1}H-NMR ($CDCl_3$) δ_H: 7.58—7.56 (2H, m, aromatic protons), 7.40—7.37 (3H, m, aromatic protons), 5.73 (1H, br d, J=5 Hz, H-2), 5.48 (1H, dd, J=2, 2 Hz, H-6), 3.56 (3H, br s, OMe), 2.17 (2H, br d, J=14 Hz, H-3eq, H-5eq), 1.83 (1H, br dd, J=14, 14 Hz, H-3ax), 1.69 (1H, m, H-4), 1.59 (3H, br s, H_3-7), 1.57 (1H, m, H-5ax), 1.18 (3H, s, H_3-9), 1.17 (3H, s, H_3-10); HR-ESI-TOF-MS (positive-ion mode) m/z: 409.1602 $[M+Na]^+$ (Calcd for $C_{20}H_{25}O_4F_3Na$: 409.1597).

Sugar Analysis for Compounds 2—5 The absolute configurations of sugars were determined according to the literature.[16] In brief, compounds **2** (0.4 mg), **3** (0.3 mg), **4** (1.0 mg), and **5** (1.1 mg) were hydrolyzed with 1 M HCl (0.2 ml) at 90 °C for 2 h. The reaction mixtures were washed with an equal amount of EtOAc (0.2 ml), and then the remaining H_2O layers were passed through ion-exchange resin Amberlite MB-3 (Organo, Japan). The filtrates were evaporated to complete dryness. The residues were each dissolved in 0.1 ml of dry pyridine, and then 0.5 mg of L-cysteine methyl ester was added. After 1 h reaction at 60 °C, 1.4 mg of *o*-tolylthioisocyanate in 70 μl of pyridine was added, followed by further reaction for 1 h at 60 °C. The reaction mixture was then directly analyzed by HPLC [ODS: Cosmosil 5C18ARII (4.6 mm×250 mm), 25% CH_3CN/50 mM H_3PO_4, 0.8 ml/min, UV detector at 250 nm] to give peaks for thiocarbamoylthiazolidine derivatives of D-glucose (17.5 min), L-arabinose (19.5 min), and L-rhamnose (30.4 min), respectively. The peaks were identified by co-chromatography with the derivatives of authentic sugars.

DPPH Radical-Scavenging Activity Assay The reagents, (*S*)-(−)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), were purchased from Aldrich Chemical Co., and the DPPH radical-scavenging activities of the isolated compounds were examined according to the previously described method.[17] In brief, the absorbance with various concentrations of the test compounds dissolved in MeOH (100 μl) in a 96-well microtiter plate was measured at 515 nm as $A_{background}$. Then, 200 μM of a DPPH solution (100 μl) was added to each well, followed by incubation for 30 min at 25 °C. The absorbance was measured again as A_{sample}. Inhibition of the DPPH radical was calculated using the following equation:

$$\text{inhibition (\%)} = [1-(A_{sample}-A_{background})/A_{DMSO}-A_{background})]\times 100$$

where A_{DMSO} is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test compounds). IC_{50} was determined as the concentration of sample required to inhibit the formation of the DPPH radical by 50%.

Human Cancer Cell Growth Inhibition Assay The growth inhibitory activities were determined using human promyelocytic leukemia cells (HL-60) and human small cell lung cancer cells (SBC-3) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method with minor modifications.[18] In brief, cells (5×10^3 cells/100 μl complete medium) were cultured in 96-well plates with different concentrations of the test compounds (50, 30, 10, 5 μM; in all the experiments, 1% of DMSO was present as a vehicle) for 72 h at 37 °C. Then the culture supernatants were replaced with 100 μl of a MTT solution comprising 0.5 mg/ml of MTT in complete medium. After 1 h incubation at 37 °C, the formazan precipitates were dissolved in DMSO and then the optical density values for each well was measured at 540 nm with a microplate reader.

The cell growth inhibition was calculated using the following equation:

$$\text{inhibition (\%)} = [1 - (A_{\text{sample}} - A_{\text{background}})/A_{\text{DMSO}} - A_{\text{background}})] \times 100$$

where A_{DMSO} is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test compounds). Etoposide was used as a positive control. IC_{50} was determined as the concentration of sample required to inhibit the formation of MTT formazan by 50%.

Acknowledgments The authors are grateful for access to the superconducting NMR instrument (JEOL ECA-600) and the Applied Biosystem QSTAR XL system ESI (nano Spray) mass spectrometer at the Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University. This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Nos. 22590006 and 23590130), and the Ministry of Health, Labour and Welfare. Thanks are also due to the Research Foundation for Pharmaceutical Sciences and the Takeda Science Foundation for the financial support.

References

1) Zhong X. N., Otsuka H., Ide T., Hirata E., Takushi A., Takeda Y., *Phytochemistry*, **46**, 943—946 (1997).
2) Otsuka H., Zhong X. N., Hirata E., Shinzato T., Takeda Y., *Chem. Pharm. Bull.*, **49**, 1093—1097 (2001).
3) Zhong X. N., Otsuka H., Ide T., Hirata E., Takushi A., Takeda Y., *Phytochemistry*, **49**, 2149—2153 (1998).
4) Zhong X. N., Otsuka H., Ide T., Hirata E., Takeda Y., *Phytochemistry*, **52**, 923—927 (1999).
5) Dong M., Nagaoka M., Miyazaki S., Iriye R., Hirota M., *Biosci. Biotechnol. Biochem.*, **63**, 1650—1653 (1999).
6) Hirota M., Miyazaki S., Minakuchi T., Takagi T., Shibata H., *Biosci. Biotechnol. Biochem.*, **66**, 655—659 (2002).
7) Kasai R., Suzuno M., Asakawa J., Tanaka O., *Tetrahedron Lett.*, **1**, 175—178 (1977).
8) Kusumi T., Ooi T., OhkuboY., Yabuuchi T., *Bull. Chem. Soc. Jpn.*, **79**, 965—980 (2006).
9) Jia Z., Koike K., Ohmoto T., Ni M., *Phytochemistry*, **37**, 1389—1396 (1994).
10) Ishii H., Kitagawa I., Matsushita K., Shirakawa K., Tori K., Tozyo T., Yoshikawa M., Yoshimura Y., *Tetrahedron Lett.*, **22**, 1529—1532 (1981).
11) Bloor S. J., Qi L., *J. Nat. Prod.*, **57**, 1354—1360 (1994).
12) Tommasi N., Piacente S., Simone F., Pizza C., *J. Nat. Prod.*, **56**, 1669—1675 (1993).
13) Chang X., Li W., Jia Z., Satou T., Fushiya S., Koike K., *J. Nat. Prod.*, **70**, 179—187 (2007).
14) Jung H. A., Kim J. E., Chung H. Y., Choi J. S., *Arch. Pharm. Res.*, **26**, 279—285 (2003).
15) Castillo-Muñoz N., Gómez-Alonso S., García-Romero E., Gómez M. V., Velders A. H., Hermosín-Gutiérrez I., *J. Agric. Food Chem.*, **57**, 209—219 (2009).
16) Tanaka T., Nakashima T., Ueda T., Tomii K., Kouno I., *Chem. Pharm. Bull.*, **55**, 899—901 (2007).
17) Matsunami K., Takamori I., Shinzato T., Aramoto M., Kondo K., Otsuka H., Takeda Y., *Chem. Pharm. Bull.*, **54**, 1403—1407 (2006).
18) Yen C. T., Wu C. C., Lee J. C., Chen S. L., Morris-Natschke S. L., Hsieh P. W., Wu Y. C., *Eur. J. Med. Chem.*, **45**, 2494—2502 (2010).