Triterpene Glycosides from *Curculigo orchioides* and Their Cytotoxic Activity

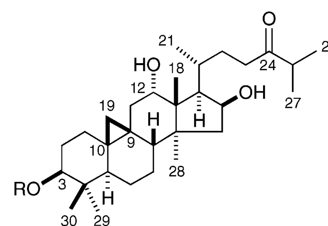
Akihito Yokosuka,*[,†] Koji Sato,[†] Takao Yamori,[‡] and Yoshihiro Mimaki*[,†]

Laboratory of Medicinal Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Horinouchi 1432-1, Hachiouji, Tokyo 192-0392, Japan, and Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-10-6, Ariake, Koutou-ku, Tokyo 135-8550, Japan

Received February 17, 2010

Six new cycloartane glycosides (**1**−**6**) were isolated from the rhizomes of *Curculigo orchioides*. The structures of **1**−**6** were determined by spectroscopic analyses and the results of hydrolytic cleavage. Compounds **1**−**6**, and their common aglycone (**1a**), were evaluated for cytotoxic activity against HL-60 human leukemia cells. Compounds **1** and **1a** showed cytotoxic activity against HL-60 cells with IC_{50} values of 9.0 and 1.8 μM, respectively. The cancer cell growth inhibition of **1a** was also examined using a panel of 39 human cancer cell lines in the Japanese Foundation for Cancer Research.

Curculigo orchioides Gaertn. (Hypoxidaceae) is a perennial plant that grows in Japan, China, India, and Australia.[1] Its rhizomes have been used in traditional medicines in tonics and for the treatment of asthma and hyperpiesia.[2] Fragmentary phytochemical examinations have been carried out on *C. orchioides*, and triterpene glycosides[3−7] and phenolic glycosides[8−11] have been isolated and identified. In this study of the rhizomes of this plant, particular attention has been paid to triterpene glycoside constituents, and six new cycloartane glycosides (**1**−**6**) were isolated. The structures were determined on the basis of spectroscopic analyses, including two-dimensional (2D) NMR spectroscopic data, and the results of hydrolytic cleavage. Compounds **1**−**6**, and their common aglycone (**1a**), were evaluated for cytotoxic activity against HL-60 human promyelocytic leukemia cells. The cancer cell growth inhibition of **1a** was also examined using a panel of 39 human cancer cell lines in the Japanese Foundation for Cancer Research.



	R
1	Glc
1a	H
2	S_1
3	S_2
4	S_3
5	S_4
6	S_5

Results and Discussion

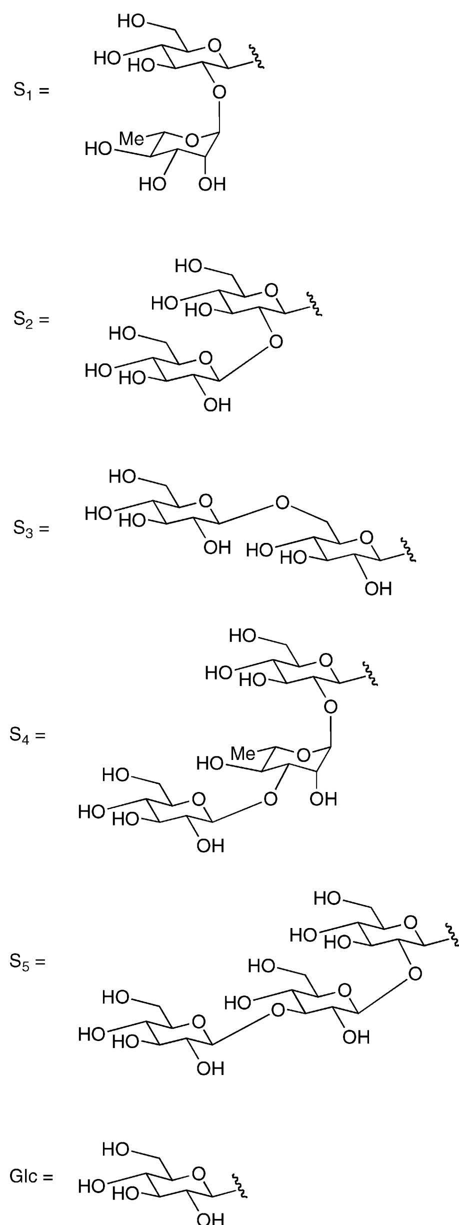
Rhizomes of *C. orchioides* were extracted with MeOH, and the crude extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc. The MeOH eluate fraction was repeatedly subjected to silica gel and octadecylsilanized (ODS) silica gel column chromatography (CC) to afford compounds **1**−**6**. The ^{1}H and ^{13}C NMR spectra of compounds **1**−**6** are compiled in Tables S1 and S2 of the Supporting Information.

Compound **1** was obtained as colorless needles, mp 215−217 °C, with the molecular formula $C_{36}H_{60}O_9$ as determined from HRESITOFMS (*m/z* 637.4302 $[M + H]^+$), ^{13}C NMR, and DEPT data. The existence of a carbonyl group was verified by the IR (1697 cm^{-1}) and ^{13}C NMR (δ 216.0) spectra. The ^{1}H NMR spectrum of **1** showed four three-proton singlets (δ 1.40, 1.36, 1.33, and 1.10), three three-proton doublets [δ 1.39 (J = 7.5 Hz), 1.03 (J = 6.9 Hz), and 1.00 (J = 6.9 Hz)], and characteristic cyclopropane methylene proton signals at δ 0.48 (1H, d, J = 3.8 Hz) and 0.32 (1H, d, J = 3.8 Hz). An anomeric proton signal for a hexose was also observed at δ 4.96 (1H, d, J = 7.8 Hz). Enzymatic hydrolysis of **1** with naringinase gave an aglycone (**1a**) and D-glucose. The identification of D-glucose, including its absolute configuration, was carried out by direct HPLC analysis of the hydrolysate using an optical rotation detector. The above data suggested that **1** was a cycloartane-type triterpene monoglucoside with a carbonyl group.

The ^{1}H NMR spectrum of **1a** ($C_{30}H_{50}O_4$) contained signals for three exchangeable protons at δ 5.84 (1H, br s), 5.54 (1H, br s), and 5.53 (1H, br s), which were removed by the addition of HCl vapor, as well as signals for cyclopropane methylene protons and seven triterpene methyl groups (Table 1). The ^{1}H−^{1}H COSY spectrum together with HMQC data revealed that **1a** had five proton spin systems, a: $-C_{(1)}H_2-C_{(2)}H_2-C_{(3)}H(-OH)-$, b: $-C_{(5)}H-C_{(6)}H_2-C_{(7)}H_2-C_{(8)}H-$, c: $-C_{(11)}H_2-C_{(12)}H(-OH)-$, d: $-C_{(15)}H_2-C_{(16)}H(-OH)-C_{(17)}H-C_{(20)}H(-C_{(21)}H_3)-C_{(22)}H_2-C_{(23)}H_2-$, and e: $-C_{(25)}H(-C_{(26)}H_3)-C_{(27)}H_3$. In the HMBC spectrum, long-range correlations between δ_H 3.54 (H-3)/1.36 (H-5) and δ_C 41.1 (C-4), δ_H 1.23 (Me-29)/1.11 (Me-30) and δ_C 78.0 (C-3)/41.1 (C-4)/47.7 (C-5), δ_H 1.61 (H-1ax)/1.30 (H-1eq) and δ_C 44.7 (C-5)/26.4 (C-10), and δ_H 1.62 (H-8) and δ_C 20.1 (C-9) showed linkages of fragment-a with an OH group at C-3 and fragment-b through the quaternary carbons C-4, C-9, and C-10 (A and B rings) and attachments of the geminal methyl groups (Me-29 and Me-30) to C-4. HMBC correlations between δ_H 2.41 (H-11a)/1.93 (H-11b) and δ_C 30.2 (C-19)/20.1 (C-9) gave evidence for a cyclopropane ring (C-9, C-10, and C-19). Further HMBC correlations between δ_H 0.54 (H-19a)/0.41 (H-19b) and δ_C 20.1 (C-9)/26.4 (C-10) and between δ_H 4.16 (H-12) and δ_C 50.1 (C-13)/18.5 (Me-18) allowed fragment-c to be placed in ring C with an OH group at C-12. Similarly, the respective linkages of C-15 and C-17 of fragment-d to the quaternary carbons C-14 and C-13, thus forming ring D and locating an OH group at C-16, were revealed by HMBC correlations between δ_H 4.84 (H-16) and δ_C 50.1 (C-13), δ_H 1.85 (H-15β) and δ_C 47.1 (C-14), δ_H 1.43 (Me-18) and δ_C 50.1 (C-13)/47.1 (C-14)/49.3 (C-17), and δ_H 1.34 (Me-28) and δ_C 49.3 (C-8)/50.1 (C-13)/47.1 (C-14) (Figure 1). Furthermore, HMBC correlartions were

* To whom correspondence should be addressed. Tel: +81-42-676-4577. Fax: +81-42-676-4579. E-mail: yokosuka@toyaku.ac.jp; mimakiy@toyaku.ac.jp.
[†] Tokyo University of Pharmacy and Life Sciences.
[‡] Japanese Foundation for Cancer Research.

10.1021/np100111s © 2010 American Chemical Society and American Society of Pharmacognosy
Published on Web 06/04/2010



observed between δ_H 2.70 (2H, H_2-23)/2.56 (H-25)/1.01 (Me-26)/1.03 (Me-27) and the carbonyl carbon at δ_C 216.0 (C-24). These data led to the assignment of the location of three OH groups at C-3, C-12, and C-16 and a carbonyl group at C-24. NOE correlations between δ_H 3.54 (H-3) and δ_H 1.61 (H-1ax)/1.36 (H-5), δ_H 1.62 (H-8) and δ_H 1.43 (Me-18)/0.54 (H-19a), and δ_H 1.34 (Me-28) and δ_H 2.78 (H-17) observed in the phase-sensitive NOESY spectrum (Figure 2) and the proton spin-coupling constants (Table 1) indicated that **1a** had the usual 9,19-cycloartane ring junctions, as shown, and the C-3β, C-12α, and C-16β configurations. The large J value between H-17 and H-20 (J = 10.9 Hz) indicated that the $H_{17}-C_{17}-C_{20}-H_{20}$ part was preferably *trans*-oriented, and an NOE correlation between H-20 and Me-18 made it possible to assign the 17β and 20S configurations. Thus, the structure of **1a** was determined to be 3β,12α,16β-trihydroxy-9,19-cyclolanostan-24-one.

The ^{13}C NMR spectrum of **1** showed the presence of a β-glucopyranosyl unit (Glc) [δ_H 4.96 (d, J = 7.8 Hz); δ_C 106.8, 75.8, 78.7, 71.8, 78.2, and 63.0]. An HMBC correlation was observed between H-1 of Glc at δ_H 4.96 and C-3 of the aglycone moiety at δ_C 88.7. Accordingly, the structure of **1** was characterized as 3β-[(β-D-glucopyranosyl)oxy]-12α,16β-dihydroxy-9,19-cyclolanostan-24-one.

Table 1. ^{1}H and ^{13}C NMR Chemical Shift Assignments of **1** and **1a** in C_5D_5N[a]

	1		**1a**	
position	^{1}H	^{13}C	^{1}H	^{13}C
1 eq	1.18 m	32.4	1.30 m	32.7
1 ax	1.51 m		1.61 m	
2 eq	2.38 m	29.9	1.96 m	31.3
2 ax	1.88 m		1.87 m	
3	3.52 dd (11.7, 4.4)	88.7	3.54 dd (11.6, 4.6)	78.0
4		41.3		41.1
5	1.32 dd (13.5, 4.3)	47.8	1.36 m	47.7
6 α	1.58 m	21.4	1.62 m	21.7
6 β	0.78 m		0.78 m	
7 α	1.14 m	26.7	1.17 m	26.8
7 β	1.38 m		1.38 m	
8	1.58 dd (12.3, 4.0)	49.3	1.62 m	49.3
9		20.0		20.1
10		26.1		26.4
11 α	2.35 dd (14.5, 5.5)	40.1	2.41 dd (14.6, 5.7)	40.3
11 β	1.86 dd (14.5, 9.3)		1.93 dd (14.6, 9.4)	
12	4.13 dd (9.3, 5.5)	72.5	4.16 dd (9.4, 5.7)	72.5
13		50.0		50.1
14		47.1		47.1
15 α	2.21 dd (13.0, 8.1)	50.3	2.24 dd (12.9, 8.0)	50.3
15 β	1.82 dd (13.0, 4.5)		1.85 dd (12.9, 4.8)	
16	4.82 ddd (8.1, 7.5, 4.5)	71.7	4.84 ddd (8.0, 7.5, 4.8)	71.7
17	2.77 dd (10.9, 7.5)	49.3	2.78 dd (10.9, 7.5)	49.3
18	1.40 s	18.5	1.43 s	18.5
19 a	0.48 d (3.8)	30.1	0.54 d (4.0)	30.2
19 b	0.32 d (3.8)		0.41 d (4.0)	
20	2.25 m	30.2	2.27 m	30.3
21	1.39 d (7.5)	17.1	1.41 d (6.7)	17.1
22 a	2.38 m	30.8	2.38 m	30.8
22 b	1.55 m		1.56 m	
23 a	2.68 m	38.1	2.70 m	38.2
23 b	2.68 m		2.70 m	
24		216.0		216.0
25	2.57 septet (6.9)	40.7	2.56 septet (6.9)	40.7
26	1.00 d (6.9)	18.4	1.01 d (6.9)	18.4
27	1.03 d (6.9)	18.4	1.03 d (6.9)	18.4
28	1.33 s	22.1	1.34 s	22.1
29	1.36 s	25.8	1.23 s	26.2
30	1.10 s	15.5	1.11 s	14.9
1′	4.96 d (7.8)	106.8		
2′	4.05 dd (8.9, 7.8)	75.8		
3′	4.25 dd (8.9, 8.9)	78.7		
4′	4.24 dd (8.9, 8.9)	71.8		
5′	3.97 ddd (8.9, 5.3, 2.5)	78.2		
6′ a	4.55 dd (11.7, 2.5)	63.0		
6′ b	4.40 dd (11.7, 5.3)			

[a] Values in parentheses are coupling constants in Hz.

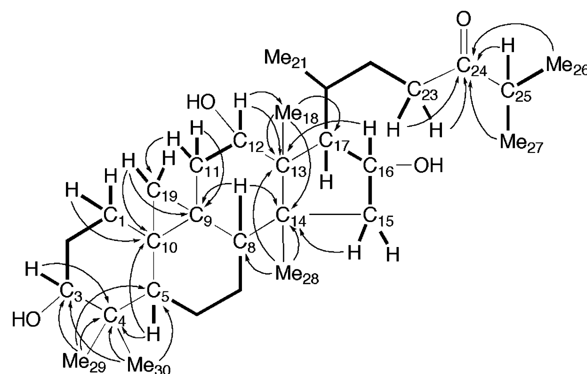


Figure 1. HMBC correlations of **1a**.Bold lines indicate the ^{1}H−^{1}H couplings, and arrows indicate ^{1}H/^{13}C long-range correlations.

Compound **2** was obtained as an amorphous solid with the molecular formula $C_{42}H_{70}O_{13}$ by HRESITOFMS, higher than that of **1** by $C_6H_{10}O_4$. The ^{1}H NMR spectrum of **2** contained signals for two anomeric protons (δ 6.57 and 4.95), a cyclopropane methylene group [δ 0.49 (1H, d, J = 3.6 Hz) and 0.30 (1H, d, J = 3.6 Hz)], four tertiary methyl groups (δ 1.38, 1.33, 1.28, and 1.25), three secondary methyl groups (δ 1.38, 1.02, and 1.00), and the

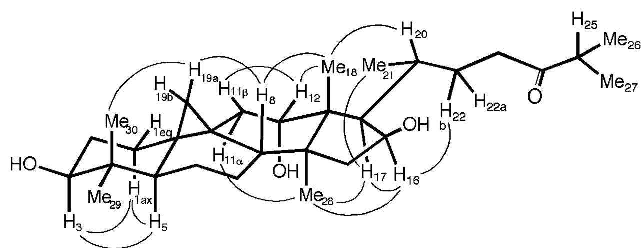


Figure 2. Important NOE correlations of **1a**.

methyl group of a 6-deoxyhexopyranosyl moiety [δ 1.72 (1H, d, J = 6.2 Hz)]. Acid hydrolysis of **2** with 0.25 M HCl gave L-rhamnose and D-glucose. The ^{13}C NMR spectrum of **2** had a set of signals corresponding to a terminal α-L-rhamnopyranosyl unit (Rha) (δ 101.7, 72.5, 72.5, 74.1, 69.6, and 18.7), and the resonance due to C-2 of the glucose unit (Glc) was attached at C-3 of the aglycone as its neighboring carbons varied. All other signals remained almost the same as those in **1**. In the HMBC spectrum, long-range correlations were observed between H-1 of Rha at δ_H 6.57 and C-2 of Glc at δ_C 77.7 and between H-1 of Glc at δ_H 4.95 and C-3 of the aglycone at δ_C 88.4. Thus, **2** was formulated as 12α,16β-dihydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]-9,19-cyclolanostan-24-one.

Compound **3** was obtained as an amorphous solid with a molecular formula of $C_{42}H_{70}O_{14}$. Acid hydrolysis of **3** gave D-glucose. Analysis of the ^{13}C NMR spectrum of **3** and comparison with that of **2** indicated that the structure of the aglycone of **3** was identical to that of **2**, but differed from **2** in terms of the monosaccharide constituent. Instead of the signals for the rhamnosyl moiety, six signals assignable to a β-D-glucopyranosyl group (Glc′) were observed at δ 106.1, 77.7, 78.0, 71.7, 78.2, and 62.8. In the HMBC spectrum, long-range correlations were observed between H-1 of Glc′ at δ_H 5.39 and C-2 of Glc at δ_C 83.5 and between H-1 of Glc at δ_H 4.94 and C-3 of the aglycone at δ_C 88.7. Thus, **3** was determined to be 3β-[(*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]-12α,16β-dihydroxy-9,19-cyclolanostan-24-one.

Compound **4** had the molecular formula $C_{42}H_{70}O_{14}$, and the ^1H and ^{13}C NMR spectra of **4** were very similar to those of **3**. However, the terminal β-D-glucopyranosyl unit (Glc′) was attached to C-6 of an inner β-D-glucopyranosyl unit (Glc) [δ_H 4.91 (d, J = 7.7 Hz); δ_C 106.8, 75.6, 78.6, 71.7, 77.1, and 70.4] instead of C-2 of Glc. Acid hydrolysis of **4** gave only D-glucose. In the HMBC spectrum of **4**, long-range correlations were observed between H-1 of Glc′ and C-6 of Glc and between H-1 of Glc and C-3 of the aglycone. Thus, **4** was formulated as 3β-[(*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)oxy]-12α,16β-dihydroxy-9,19-cyclolanostan-24-one.

Compound **5** had the molecular formula $C_{48}H_{80}O_{18}$, higher than that of **2** by $C_6H_{10}O_5$. Although the ^1H and ^{13}C NMR spectra of the aglycone moiety of **5** and **2** were similar, the ^1H NMR spectrum of **5** contained resonances for three anomeric protons [δ 6.53 (br s, Rha), 5.51 (d, J = 7.9 Hz, Glc′), and 4.94 (d, J = 7.0 Hz, Glc)]. Acid hydrolysis of **5** gave L-rhamnose and D-glucose. On comparison of the ^{13}C NMR spectrum for the sugar moiety of **5** with that of **2**, a set of six additional signals corresponding to a terminal β-D-glucopyranosyl unit (Glc′) was observed, and the signal assignable to C-3 of Rha was significantly shifted downfield. HMBC correlations between H-1 of Glc′ and C-3 of Rha, H-1 of Rha and C-2 of Glc, and H-1 of Glc and C-3 of the aglycone were detected. Thus, **5** was characterized as 3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]-12α,16β-dihydroxy-9,19-cyclolanostan-24-one.

The molecular formula of compound **6** was deduced as $C_{48}H_{80}O_{19}$ on the basis of HRESITOFMS, which was higher than that of **3** by $C_6H_{10}O_5$. The ^1H and ^{13}C NMR spectra of **6** indicated that it was a triglucoside of **1a**. In the HMBC spectrum of **6**, long-range correlations were observed between H-1 of Glc″ and C-3 of Glc′, H-1 of Glc′ and C-2 of Glc, and H-1 of Glc and C-3 of the aglycone. Accordingly, **6** was formulated as 3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]-12α,16β-dihydroxy-9,19-cyclolanostan-24-one.

The new glycosides (**1**−**6**) and their common aglycone (**1a**) were each evaluated for cytotoxic activity against HL-60 cells. Compounds **1** and **1a** showed cytotoxic activity against HL-60 cells with IC_{50} values of 9.0 and 1.8 μM, respectively, while etoposide, used as a positive control, gave an IC_{50} value of 0.39 μM. Compounds **2**−**6** were not cytotoxic to HL-60 cells at sample concentrations of 20 μM.

The cancer cell growth inhibitory property of **1a** was evaluated using a disease-orientated panel composed of 39 human cancer cell lines (HCC panel) in the Japanese Foundation for Cancer Research.[12] Compound **1a** was cytotoxic against all of the cell lines tested at considerably low concentrations, and the average logarithm of the GI_{50} (MG-MID) was −5.51 (Table S3, Supporting Information). The central nervous system cancer SF-268 (log GI_{50} −5.86), melanoma LOX-IMVI (log GI_{50} −5.81), and ovarian cancer OVCAR-8 (log GI_{50} −5.81) cell lines were especially sensitive to **1a**.

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. Melting points were measured on a YANACO micro melting point apparatus (Kyoto, Japan). NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^1H NMR, Karlsruhe, Germany) and a Bruker DRX-400 (400 MHz for ^1H NMR) spectrometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. HRESITOFMS was recorded on a Waters-Micromass LCT mass spectrometer (Manchester, UK). Diaion 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 silica gel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP_{18} F_{254S} plates (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a PX-8010 controller (Tosoh), a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and a Rheodyne injection port. The following materials and reagents were used for cell culture and the assay of cytotoxic activity: Spectra Classic microplate reader (Tecan, Salzburg, Austria); 96-well flat bottom plates (Iwaki Glass, Chiba, Japan); JCRB 0085 HL-60 cells (Human Science Research Resources Bank, Osaka, Japan); RPMI 1640 medium, etoposide, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO); fetal bovine serum (FBS) (Bio-Whittaker, Walkersville, MO); and penicillin G sodium salt and streptomycin sulfate (Meiji-Seika, Tokyo, Japan). All other chemicals used were of biochemical reagent grade.

Plant Material. The rhizomes of *Curculigo orchioides* were collected in fields near Beijing, China, in 2002. The plant was identified by Dr. Yutaka Sashida, emeritus professor of medicinal pharmacognosy at Tokyo University of Pharmacy and Life Sciences. A voucher specimen has been deposited in our laboratory (voucher no. CO-2002-001, Laboratory of Medicinal Pharmacognosy).

Extraction and Isolation. The plant material (fresh weight, 2.9 kg) was extracted with hot MeOH twice (each 6 L). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (220 g) was passed through a Diaion HP-20 column (90 mm i.d. × 300 mm), successively eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc (each 15 L). Column chromatography of the MeOH eluate portion (30 g) on silica gel (75 mm i.d. × 200 mm) and elution with a stepwise gradient mixture of $CHCl_3$−MeOH−H_2O (9:1:0; 40:10:1; 30:10:1; 20:10:1; 1:1:0) and finally with MeOH alone gave nine fractions (I−IX). Fraction III was chromatographed on ODS silica gel (60 mm i.d. × 200 mm) eluted with MeOH−H_2O (8:3; 8:1) and silica gel (26 mm i.d. × 200 mm) with EtOAc−MeOH−H_2O (20:2:1) to give **1** (30.0 mg). Fraction V was suspended in MeOH, and the insoluble

Table 2. ^{1}H and ^{13}C NMR Chemical Shift Assignments for the Sugar Moiety of Compounds **2**−**6** in $C_5D_5N^a$

	2			3			4			5			6	
	^{1}H	^{13}C		^{1}H	^{13}C		^{1}H	^{13}C		^{1}H	^{13}C		^{1}H	^{13}C
Glc 1	4.95 d (7.2)	105.2	Glc 1	4.94 d (7.6)	104.9	Glc 1	4.91 d (7.7)	106.8	Glc 1	4.94 d (7.0)	105.3	Glc 1	4.90 d (7.5)	104.7
2	4.30 dd (9.3, 7.2)	77.7	2	4.24 dd (9.0, 7.6)	83.5	2	4.00 dd (9.2, 7.7)	75.6	2	4.25 dd (9.1, 7.0)	76.9	2	4.22 dd (9.1, 7.5)	82.8
3	4.28 dd (9.3, 9.3)	79.9	3	4.32 dd (9.0, 9.0)	78.4	3	4.20 dd (9.2, 9.2)	78.6	3	4.23 dd (9.1, 9.1)	79.7	3	4.27 dd (9.1, 9.1)	78.4
4	4.15 dd (9.3, 9.3)	72.1	4	4.17 dd (9.0, 9.0)	71.6	4	4.13 dd (9.2, 9.2)	71.7	4	4.16 dd (9.1, 9.1)	72.1	4	4.14 dd (9.1, 9.1)	71.5
5	3.92 ddd (9.3, 5.3, 2.3)	78.1	5	3.88 ddd (9.0, 4.8, 2.3)	78.0	5	4.12 m	77.1	5	3.91 ddd (9.1, 5.0, 2.2)	78.1	5	3.86 ddd (9.1, 5.5, 2.2)	77.9
6a	4.53 dd (11.7, 2.3)	62.8	6a	4.48 dd (12.0, 2.3)	62.8	6a	4.86 m	70.4	6a	4.52 dd (11.8, 2.2)	62.8	6a	4.50 dd (11.7, 2.2)	62.7
6b	4.35 dd (11.7, 5.3)		6b	4.35 dd (12.0, 5.3)		6b	4.34 dd (11.1, 4.2)		6b	4.35 dd (11.8, 5.0)		6b	4.34 dd (11.7, 5.5)	
Rha 1	6.57 br s	101.7	Glc′ 1	5.39 d (7.6)	106.1	Glc′ 1	5.14 d (7.8)	105.5	Rha 1	6.53 br s	101.3	Glc′ 1	5.40 d (7.6)	105.2
2	4.86 br d (3.4)	72.5	2	4.14 dd (9.1, 7.6)	77.7	2	4.06 dd (8.8, 7.8)	75.2	2	5.04 m	71.7	2	4.13 dd (9.3, 7.6)	75.7
3	4.71 dd (9.3, 3.4)	72.5	3	4.25 dd (9.1, 9.1)	78.0	3	4.24 dd (8.8, 8.8)	78.4	3	4.89 dd (9.5, 2.9)	83.3	3	4.21 dd (9.3, 9.3)	88.0
4	4.35 dd (9.3, 9.3)	74.1	4	4.34 dd (9.1, 9.1)	71.7	4	4.25 dd (8.8, 8.8)	71.7	4	4.53 dd (9.5, 9.5)	73.0	4	4.21 dd (9.3, 9.3)	69.7
5	4.83 dq (9.3, 6.2)	69.6	5	3.94 ddd (9.1, 4.0, 3.6)	78.2	5	3.94 ddd (8.8, 5.1, 1.9)	78.4	5	4.81 dq (9.5, 6.1)	69.5	5	3.85 ddd (9.3, 4.5, 2.5)	77.9
6	1.72 d (6.2)	18.7	6a	4.51 dd (11.5, 3.6)	62.8	6a	4.52 dd (11.8, 1.9)	62.8	6	1.64 d (6.1)	18.6	6a	4.43 dd (11.5, 2.5)	62.4
			6b	4.47 dd (11.5, 4.0)		6b	4.38 dd (11.8, 5.1)					6b	4.35 dd (11.5, 4.5)	
									Glc′ 1	5.51 d (7.9)	106.8	Glc″ 1	5.28 d (7.9)	105.7
									2	4.12 dd (9.2, 7.9)	75.9	2	4.07 dd (9.3, 7.9)	75.7
									3	4.28 dd (9.2, 9.2)	78.5	3	4.25 dd (9.3, 9.3)	78.2
									4	4.30 dd (9.2, 9.2)	71.3	4	4.19 dd (9.3, 9.3)	71.6
									5	3.99 ddd (9.2, 4.6, 2.2)	78.6	5	4.03 ddd (9.3, 5.8, 2.6)	78.6
									6a	4.48 dd (11.7, 2.2)	62.4	6a	4.54 dd (11.6, 2.6)	62.5
									6b	4.39 dd (11.7, 4.6)		6b	4.31 dd (11.6, 5.8)	

[a] Values in parentheses are coupling constants in Hz.

solid was filtered to give **2** (97.7 mg). Fraction VI was separated by an ODS silica gel column (60 mm i.d. × 200 mm) eluted with MeCN−H_2O (4:7; 2:3) to give five subfractions (VIa−VIe). Fraction VIc was subjected to a silica gel column (26 mm i.d. × 200 mm) eluted with $CHCl_3$−MeOH−H_2O (40:10:1) and an ODS silica gel column (30 mm i.d. × 170 mm) with MeOH−H_2O (16:9) to afford **3** (17.0 mg). Fraction VId was subjected to CC on silica gel (26 mm i.d. × 200 mm) eluted with $CHCl_3$−MeOH−H_2O (40:10:1) and ODS silica gel (20 mm i.d. × 170 mm) with MeOH−H_2O (16:9) to give **4** (9.6 mg). Fraction VII was subjected to silica gel CC (28 mm i.d. × 200 mm) eluted with $CHCl_3$−MeOH−H_2O (40:10:1) and an ODS silica gel column (28 mm i.d. × 170 mm) with MeCN−H_2O (1:2) and MeOH−H_2O (16:9) to afford **5** (138 mg). Fraction VIII was separated using an ODS silica gel column (60 mm i.d. × 200 mm) eluted with MeOH−H_2O (4:3; 8:5; 2:1) to give six subfractions (VIIIa−VIIIf). Fraction VIIId was subjected to silica gel CC (42 mm i.d. × 200 mm) eluted with $CHCl_3$−MeOH−H_2O (30:10:1) and ODS silica gel CC (20 mm i.d. × 170 mm) with MeCN−H_2O (2:5) to give **6** (98.7 mg).

Compound 1: colorless needles (hexane−Me_2O−MeOH, 1:5:5); mp 215−217 °C; $[\alpha]_D^{29}$ +40.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3389 (OH), 2931 and 2878 (CH), 1697 (C=O), 1026 (C−O) cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N), see Table 1; ^{13}C NMR (125 MHz, C_5D_5N), see Table 1; HRESITOFMS *m/z* 637.4302 $[M + H]^+$ (calcd for $C_{36}H_{61}O_9$, 637.4316).

Enzymatic Hydrolysis of 1. Compound **1** (6.3 mg) was treated with naringinase (EC 232-962-4, Sigma, 13.9 mg) in HOAc/KOAc buffer (pH 4.3, 5 mL) at room temperature for 144 h. The reaction mixture was diluted with H_2O (3.0 mL) and extracted with EtOAc (3.0 mL × 3). After concentration of the EtOAc-soluble phase, it was chromatographed on silica gel eluted with hexane−Me_2CO (10:3) to yield **1a** (2.6 mg). The H_2O-soluble phase was chromatographed on silica gel eluted with $CHCl_3$−MeOH−H_2O (7:4:1) to yield a sugar fraction (1.1 mg). The sugar fraction was analyzed by HPLC under the following conditions: column, Capcell Pak NH2 SG80 Å (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN−H_2O (17:3); flow rate, 1.0 mL/min; detection, OR. Identification of D-glucose present in the sugar fraction was carried out by comparison of the retention time and optical rotation with that of authentic sample. t_R (min): 13.02 (D-glucose, positive optical rotation).

Compound 1a: amorphous solid; $[\alpha]_D^{30}$ +40.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3379 (OH), 2928 and 2878 (CH), 1698 (C=O), 1020 (C−O) cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N), Table 1; ^{13}C NMR (125 MHz, C_5D_5N), see Table 1; HRESITOFMS *m/z* 497.3590 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3607).

Compound 2: amorphous solid; $[\alpha]_D^{28}$ −4.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3381 (OH), 2929 and 2878 (CH), 1698 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.81 (1H, m, H-16), 4.12 (1H, dd, *J* = 9.3, 5.7 Hz, H-12), 3.45 (1H, dd, *J* = 11.6, 4.2 Hz, H-3), 1.38 (3H, d, *J* = 6.7 Hz, Me-21), 1.38 (3H, s, Me-18), 1.33 (3H, s, Me-28), 1.28 (3H, s, Me-29), 1.25 (3H, s, Me-30), 1.02 (3H, d, *J* = 6.9 Hz, Me-27), 1.00 (3H, d, *J* = 6.9 Hz, Me-26), 0.49 (1H, d, *J* = 3.6 Hz, H-19a), 0.30 (1H, d, *J* = 3.6 Hz, H-19b), signals for the sugar moiety, see Table 2; ^{13}C NMR (125 MHz, C_5D_5N), signals for the aglycone moiety agreed with those of **1** within ±0.3 ppm, signals for the sugar moiety, see Table 2; HRESITOFMS *m/z* 805.4786 $[M + Na]^+$ (calcd for $C_{42}H_{70}O_{13}Na$, 805.4714).

Acid Hydrolysis of 2. A solution of **2** (3.0 mg) in 0.25 M HCl (dioxane−H_2O, 7:1, 2 mL) was heated at 95 °C for 1.5 h under an Ar atomosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and was then passed through a Sep-Pak C_{18} cartridge eluted with 30% MeOH to yield a sugar fraction (1.0 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-rhamnose and D-glucose. t_R (min): 6.75 (L-rhamnose, negative optical rotation), 13.23 (D-glucose, positive optical rotation).

Compound 3: amorphous solid; $[\alpha]_D^{30}$ −8.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3420 (OH), 2966, 2930, and 2878 (CH), 1698 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.82 (1H, ddd, *J* = 8.0, 7.6, 5.2 Hz, H-16), 4.13 (1H, dd, *J* = 9.0, 5.5 Hz, H-12), 3.44 (1H, dd, *J* = 11.6, 4.3 Hz, H-3), 1.41 (3H, s, Me-18), 1.39 (3H, d, *J* = 6.8 Hz, Me-21), 1.34 (3H, s, Me-29), 1.32 (3H, s, Me-28), 1.18 (3H, s, Me-30), 1.03 (3H, d, *J* = 6.9 Hz, Me-27), 1.01 (3H, d, *J* = 6.9 Hz, Me-26), 0.48 (1H, d, *J* = 3.8 Hz, H-19a), 0.31 (1H, d, *J* = 3.8 Hz, H-19b), see Table 2; ^{13}C NMR (125 MHz, C_5D_5N), signals for the aglycone moiety agreed with those of **1** within ±0.1 ppm, signals for the sugar moiety,

see Table 2; HRESITOFMS *m/z* 821.4659 [M + Na]$^+$ (calcd for $C_{42}H_{70}O_{14}Na$, 821.4663).

Compound 4: amorphous solid; $[\alpha]_D^{30}$ +4.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3393 (OH), 2960, 2931, and 2874 (CH), 1701 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.83 (1H, ddd, J = 8.0, 7.6, 4.5 Hz, H-16), 4.10 (1H, dd, J = 9.4, 5.6 Hz, H-12), 3.51 (1H, dd, J = 11.6, 4.4 Hz, H-3), 1.40 (3H, d, J = 6.7 Hz, Me-21), 1.39 (3H, s, Me-18), 1.32 (3H, s, Me-29), 1.30 (3H, s, Me-28), 1.08 (3H, s, Me-30), 1.03 (3H, d, J = 6.9 Hz, Me-27), 1.01 (3H, d, J = 6.9 Hz, Me-26), 0.45 (1H, d, J = 3.9 Hz, H-19a), 0.32 (1H, d, J = 3.9 Hz, H-19b), signals for the sugar moiety, see Table 2; ^{13}C NMR (125 MHz, C_5D_5N), signals for the aglycone moiety agreed with those of **1** within ±0.4 ppm, signals for the sugar moiety, see Table 2; HRESITOFMS *m/z* 821.4650 [M + Na]$^+$ (calcd for $C_{42}H_{70}O_{14}Na$, 821.4663).

Compound 5: amorphous solid; $[\alpha]_D^{30}$ −4.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3413 (OH), 2966, 2933, and 2878 (CH), 1695 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.81 (1H, m, H-16), 4.11 (1H, dd, J = 9.4, 5.8 Hz, H-12), 3.45 (1H, dd, J = 11.6, 4.2 Hz, H-3), 1.43 (3H, s, Me-29), 1.38 (3H, d, J = 6.7 Hz, Me-21), 1.38 (3H, s, Me-18), 1.32 (3H, s, Me-28), 1.28 (3H, s, Me-30), 1.02 (3H, d, J = 6.9 Hz, Me-27), 1.00 (3H, d, J = 6.9 Hz, Me-26), 0.45 (1H, d, J = 3.5 Hz, H-19a), 0.29 (1H, d, J = 3.5 Hz, H-19b), signals for the sugar moiety, see Table 2; ^{13}C NMR (125 MHz, C_5D_5N), signals for the aglycone moiety agreed with those of **1** within ±0.3 ppm, signals for the sugar moiety, see Table 2; HRESITOFMS *m/z* 945.5399 [M + H]$^+$ (calcd for $C_{48}H_{81}O_{18}$, 945.5423).

Compound 6: amorphous solid; $[\alpha]_D^{31}$ +16.0 (*c* 0.10; MeOH); IR (film) ν_{max} 3415 (OH), 2966, 2931, and 2878 (CH), 1696 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, C_5D_5N) δ 4.82 (1H, ddd, J = 8.0, 7.6, 4.7 Hz, H-16), 4.12 (1H, dd, J = 9.1, 5.9 Hz, H-12), 3.41 (1H, dd, J = 11.6, 4.4 Hz, H-3), 1.40 (3H, s, Me-18), 1.39 (3H, d, J = 6.9 Hz, Me-21), 1.32 (3H, s, Me-28), 1.30 (3H, s, Me-29), 1.16 (3H, s, Me-30), 1.03 (3H, d, J = 6.9 Hz, Me-27), 1.00 (3H, d, J = 6.9 Hz, Me-26), 0.48 (1H, d, J = 3.9 Hz, H-19a), 0.30 (1H, d, J = 3.9 Hz, H-19b), signals for the sugar moiety, see Table 2; ^{13}C NMR (125 MHz, C_5D_5N), signals for the aglycone moiety agreed with those of **1** within ±0.2 ppm, signals for the sugar moiety, see Table 2; HRESITOFMS *m/z* 961.5375 [M + H]$^+$ (calcd for $C_{48}H_{81}O_{19}$, 961.5372).

Acid Hydrolysis of 3−6. Compounds **3** (2.2 mg), **4** (2.5 mg), **5** (3.5 mg), and **6** (2.5 mg) were independently subjected to acid hydrolysis as described for **2** to give sugar fractions (**3**: 0.6 mg, **4**: 0.8 mg, **5**: 1.2 mg, **6**: 1.1 mg). HPLC analysis of the sugar fractions under the same conditions as in the case of **1** showed the presence of D-glucose and/or L-rhamnose and D-glucose in compounds **3**−**6**.

Cell Culture and Assay for Cytotoxic Activity against HL-60 Cells. HL-60 human leukemia cells were maintained in the RPMI 1640 medium containing 10% heat-inactivated FBS and antibiotics (100 units/mL penicillin sodium salt and 100 μg/mL streptomycin sulfate) in a 5% CO_2 humidified incubator at 37 °C. The cells were washed and suspended in the medium to 4 × 10^4 cells/mL, and 196 μL of this cell suspension was divided into 96-well flat bottom plates. The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After incubation, 4 μL of EtOH−H_2O (1:1) solution containing the sample was added to give the final concentrations of 0.01−20 μg/mL, and 4 μL of EtOH−H_2O (1:1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated using a modified MTT reduction assay.[13] At the end of incubation, 10 μL of 5 mg/mL MTT in phosphate-buffered saline (PBS) was added to every well, and the plate was further incubated in 5% CO_2/air for 4 h at 37 °C. Then the plate was centrifuged at 1500*g* for 5 min to precipitate MTT formazan. An aliquot of 150 μL of supernatant was removed from each well, and 175 μL of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed on a microplate mixer for 10 min and then read on a microplate reader at 550 nm. Each assay was done in triplicate, and cytotoxicity was expressed as IC_{50} value (reduction of viable cells by 50%).

Human Cancer Cell Line Panel Assay. Evaluation of **1a** for cell growth inhibition was conducted according to the method of the National Cancer Institute,[14] with modification. The cells were plated at proper density in 96-well plates in RPMI 1640 medium with 5% FBS and allowed to attach overnight. The cells were exposed to **1a** for 48 h. Then, the cell growth was determined according to the sulforhodamine B assay.[15]

Supporting Information Available: Tables of the ^{1}H and ^{13}C NMR chemical shift assignments of **1**, **1a**, and **2**−**6**, including the aglycone portion. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Tsukamoto, Y., Ed. *The Grand Dictionary of Horticulture*; Shogakukan: Tokyo, 1988; Vol. 2, pp 161−162.

(2) Jiangsu New Medicinal College. *The Dictionary of Chinese Herbal Medicines*; Shanghai Scientific and Technological Press: Shanghai, 1977; Vol. 2, pp 1364−1365.

(3) Misra, T. N.; Singh, R. S.; Tripathi, D. M.; Sharma, S. C. *Phytochemistry* **1990**, *29*, 929–931.

(4) Xu, J. P.; Xu, R. S.; Li, X. Y. *Phytochemistry* **1992**, *31*, 233–236.

(5) Xu, J. P.; Xu, R. S.; Li, X. Y. *Planta Med.* **1992**, *58*, 208–210.

(6) Xu, J. P.; Xu, R. S. *Phytochemistry* **1992**, *31*, 2455–2458.

(7) Palazzinoa, G.; Galeffi, C.; Federicia, E.; Monacheb, F. D.; Cometac, M. F.; Palmery, M. *Phytochemistry* **2000**, *55*, 411–417.

(8) Kubo, M.; Namba, K.; Nagamoto, N.; Nagao, T.; Nakanishi, J.; Uno, H.; Nishimura, H. *Planta Med.* **1983**, *47*, 52–55.

(9) Garg, S. N.; Misra, L. N.; Agarwal, S. K. *Phytochemistry* **1989**, *28*, 1771–1772.

(10) Xu, J. P.; Xu, R. S. *Acta Pharm. Sin.* **1992**, *27*, 353–357.

(11) Gupta, M.; Achari, B.; Pal, B. C. *Phytochemistry* **2005**, *66*, 659–663.

(12) Yamori, T.; Matsunaga, A.; Saito, S.; Yamazaki, K.; Komi, A.; Ishizu, K.; Mita, I.; Edatsugi, H.; Matsuba, Y.; Takezawa, K.; Nakanishi, O.; Kohno, H.; Nakajima, Y.; Komatsu, H.; Andoh, T.; Tsuruo, T. *Cancer Res.* **1999**, *59*, 4042–4049.

(13) Sargent, J. M.; Taylor, C. G. *Br. J. Cancer* **1989**, *60*, 206–210.

(14) Boyd, M. R. *Cancer Principles and Practice of Oncology Update*; Lippincott: Philadelphia, 1989; pp 1−12.

(15) Skehan, P.; Storeng, R.; Scuderio, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenny, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

NP100111S