

Triterpene Glycosides from the Underground Parts of *Caulophyllum thalictroides*

Yukiko Matsuo,^{*,†} Kazuki Watanabe,[‡] and Yoshihiro Mimaki^{*,†}

School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1, Horinouchi, Hachioji, Tokyo 192-0392, Japan, and Faculty of Pharmaceutical Sciences, Aomori University, 2-3-1, Koubata, Aomori, Aomori 030-0943, Japan

Received March 10, 2009

A total of 22 triterpene glycosides, including 10 new compounds (**1–10**), were isolated from the underground parts of *Caulophyllum thalictroides*. The structures of the new glycosides were determined on the basis of extensive spectroscopic analyses, including two-dimensional (2D) NMR data, and of hydrolytic cleavage followed by chromatographic or spectroscopic analyses. All 22 compounds were evaluated for cytotoxicity against HL-60 human leukemia cells. The triterpene monodesmosides based on oleanolic acid (**1** and **11–16**) showed cytotoxic activity against HL-60 cells with IC₅₀ values that ranged from 3.4 to 15.9 μg/mL.

Caulophyllum thalictroides (L.) Michx (Berberidaceae) is a perennial plant that grows in the United States and Canada. This plant is called Blue Cohosh and is well-known as a traditional women's herb to ease childbirth and to treat uterine inflammation among Native Americans. Extracts of the underground parts of *C. thalictroides* are used as an herbal dietary supplement for treatment of emmenagogue, rheumatism, and women's diseases and as an antispasmodic.¹ Fragmentary phytochemical studies have been carried out on *C. thalictroides*, and alkaloids (caulophyllumines A, caulophyllumines B,^{2,3} and magnoflorine⁴), steroidal glycosides (caulosaponin and caulophyllosaponin⁵), and triterpene glycosides⁶ have been isolated and identified. The present investigation of the underground parts of *C. thalictroides*, with particular attention paid to the triterpene glycoside constituents, resulted in the isolation of 22 triterpene glycosides (**1–22**), including 10 new compounds (**1–10**). This paper reports the structural determination of the new glycosides on the basis of extensive spectroscopic analyses, including two-dimensional (2D) NMR, and of hydrolytic cleavage followed by chromatographic or spectroscopic analyses. The compounds were all evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells.

Results and Discussion

The dry underground parts of *C. thalictroides* (4.0 kg) were extracted with hot MeOH. The MeOH extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column, and the MeOH-eluted fraction was subjected to column chromatography (CC) using silica gel and octadecylsilylanized (ODS) silica gel, and to reversed-phase preparative HPLC, giving compounds **1–22**. Compounds **11–22** were identified as 3β-[(α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**11**),⁷ 3β-[(α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid (**12**),⁸ 3β-[(O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**13**),⁹ 3β-[(O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid (**14**),⁶ 3β-[(α-L-arabinopyranosyl)oxy]-16α-hydroxyolean-12-en-28-oic acid (**15**),¹⁰ 3β-[(O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-16α-hydroxyolean-12-en-28-oic acid (**16**),¹¹ 3β-[(α-L-arabinopyranosyl)oxy]-16α,23-dihydroxyolean-12-en-28-oic acid (**17**),¹² 3β-[(α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**18**),¹³ 3β-[(α-L-arabinopyranosyl)oxy]-16α,23-dihydroxy-

droxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**19**),⁶ 3β-[(O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**20**),¹⁴ 3β-[(O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**21**),⁶ and 3β,23-dihydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**22**),¹⁵ respectively.

Compound **1** was obtained as an amorphous solid, [α]_D +50.0 in MeOH, with a molecular formula of C₃₅H₅₄O₈, which was assigned on the basis of the HRESITOFMS (*m/z* 603.3943 [M + H]⁺, calcd 603.3897) and ¹³C NMR data (35 carbon signals). The IR spectrum of **1** suggested the presence of hydroxy (3390 cm⁻¹) and carbonyl groups (1717 and 1692 cm⁻¹). The ¹H NMR spectrum showed signals for six tertiary methyl groups (δ 1.27 × 2, 0.99, 0.94, 0.93, and 0.82), an olefinic proton at δ 5.45 (t-like, *J* = 3.0 Hz), and an anomeric proton at δ 4.65 (d, *J* = 7.0 Hz). In the ¹³C NMR spectrum, signals at δ 180.1 (C), 144.8 (C) and 122.1 (CH), and 105.4 (CH) were assigned to a carboxy carbonyl carbon, a pair of olefinic carbons, and an anomeric carbon, respectively. These spectroscopic data suggested that **1** was a triterpene monoglycoside having a structure closely related to that of **12**. However, signals for an aldehyde group were observed at δ_H 9.73 (1H, s) and δ_C 207.0 (CH) in the ¹H and ¹³C NMR spectra of **1** instead of signals for the hydroxymethyl group of H₂-23/C-23 at δ_H 4.29 and 3.82 (ABq, *J* = 12.4 Hz) and δ_C 65.0 in **12**. Reduction of **1** with NaBH₄ in MeOH gave **12**. Thus, **1** was determined to be 3β-[(α-L-arabinopyranosyl)oxy]-23-oxo-olean-12-en-28-oic acid.

Compound **2** had the molecular formula C₃₅H₅₄O₉ as determined by HRESITOFMS (*m/z* 641.3714 [M + Na]⁺) and ¹³C NMR data. Although the ¹H and ¹³C NMR spectra of **2** were very similar to those of **1**, compound **2** had one additional oxygen atom. When the ¹³C NMR spectrum of **2** was compared with that of **1**, the C-16 methylene carbon signal (δ 23.7) in **1** was displaced by a hydroxymethine signal at δ 74.6 in **2**. The C-16 carbon signal was associated with the one-bond coupled proton resonance at δ 5.22 (H-16) in the HMQC spectrum of **2**, which was shown to be coupled with the H₂-15 protons (*J* values of less than 5.0 Hz) in the ¹H NMR spectrum. These NMR data indicated the presence of a C-16α hydroxy group in **2**. Treatment of **2** with NaBH₄ in MeOH furnished **17**. Thus, compound **2** was formulated as 3β-[(α-L-arabinopyranosyl)oxy]-16α-hydroxy-23-oxo-olean-12-en-28-oic acid.

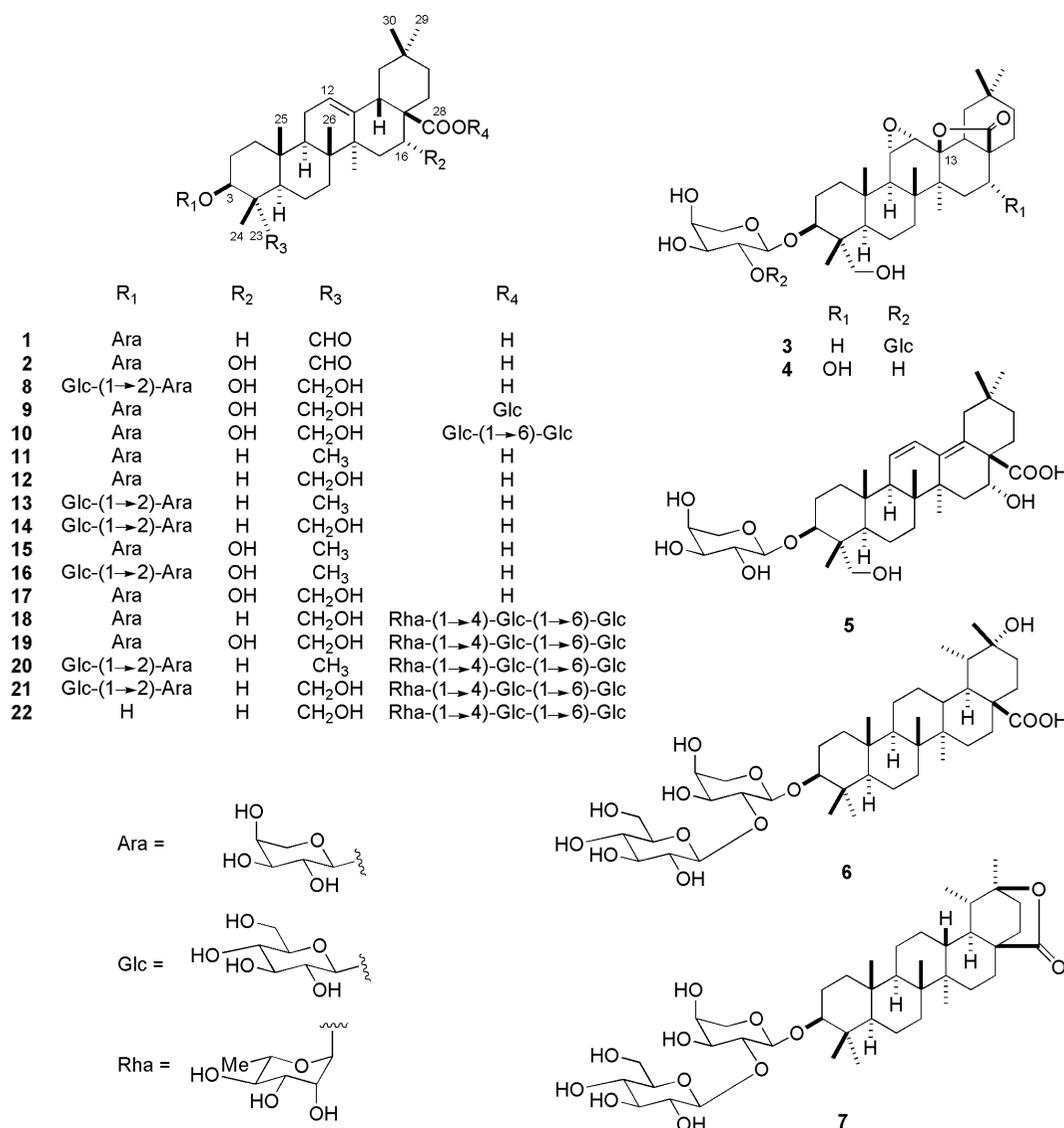
Compound **3** was obtained as an amorphous solid. Its molecular formula was derived as C₄₁H₆₄O₁₄ from the HRESITOFMS data, showing an [M + H]⁺ ion at *m/z* 781.4406, and the ¹³C NMR spectroscopic data. The ¹H NMR spectrum exhibited signals for

* To whom correspondence should be addressed. Tel: +81-426-76-4573. Fax: +81-42-676-4579. E-mail: matsuo@toyaku.ac.jp; mimaki@toyaku.ac.jp.

[†] Tokyo University of Pharmacy and Life Sciences.

[‡] Aomori University.

Chart 1



six tertiary methyl groups (δ 1.15, 1.13, 1.01, 0.99, 0.86, and 0.78), which were characteristic of triterpenes with the oleanane skeleton, as well as signals for an oxymethine proton at δ 4.14 (dd, J = 11.9, 4.7 Hz, H-3), a hydroxymethyl group at δ 4.24 and 3.73 (each 1H, d, J = 10.9 Hz, H₂-23), and anomeric protons at δ 5.18 (d, J = 7.7 Hz) and 5.15 (d, J = 6.1 Hz). In addition, a five-membered lactone ring was suggested to be included in the structure of **3** by the IR (1774 cm⁻¹) and ¹³C NMR (δ 179.0) spectra, and an epoxy ring was indicated by the ¹H NMR signals at δ 3.23 (br d, J = 3.8 Hz, H-11) and 3.10 (dd, J = 3.8, 1.3 Hz, H-12). Enzymatic hydrolysis of **3** with naringinase gave a known triterpenoid, identified as 11 α ,12 α -epoxy-3 β ,13 β ,23-dihydroxyoleanan-28-oic acid γ -lactone (**3a**; C₃₀H₄₆O₅),¹⁶ L-arabinose, and D-glucose. Identification of L-arabinose and D-glucose was by HPLC analysis of the hydrolysate. Thus, **3** was shown to be a triterpene diglycoside whose sugar moiety was composed of a β -D-glucopyranosyl unit (Glc) and an α -L-arabinopyranosyl unit (Ara). In the HMBC spectrum of **3**, long-range correlations were observed between the anomeric proton (H-1) of Glc at δ 5.18 and C-2 of Ara at δ 81.3, and between H-1 of Ara at δ 5.15 and C-3 of the aglycone at δ 81.8. Accordingly, **3** was determined to be 3 β -[(α -L-arabinopyranosyl)-(1→2)- β -D-glucopyranosyl]oxy-11 α ,12 α -epoxy-13 β ,23-dihydroxyoleanan-28-oic acid γ -lactone.

Compound **4** had the molecular formula C₃₅H₅₄O₁₀ on the basis of HRESITOFMS (m/z 657.3610 [M + Na]⁺) and ¹³C NMR data. Enzymatic hydrolysis of **4** with naringinase gave an aglycone (**4a**;

C₃₀H₄₆O₆) and L-arabinose. Analyses of the ¹H and ¹³C NMR spectra of **4a** and comparison with those of **3a** showed that the C-16 methylene carbon signal, which was observed at δ 21.6 in **3a**, was displaced by a hydroxymethine carbon signal at δ 72.2 in **4a** and that the H-16 hydroxymethine proton at δ 4.48 (br d, J = 5.0 Hz) exhibited spin-couplings with the H₂-15 methylene protons at δ 2.04 (dd, J = 14.6, 5.0 Hz, H-15ax) and 1.57 (br d, J = 14.6 Hz, H-15eq). The above data indicated that **4a** was the C-16 α hydroxy derivative of **3a**. The structure of **4a** was thus elucidated as 11 α ,12 α -epoxy-3 β ,13 β ,16 α ,23-tetrahydroxyoleanan-28-oic acid γ -lactone. In the HMBC spectrum of **4**, H-1 of an α -L-arabinopyranosyl group at δ 4.98 (d, J = 7.0 Hz) was correlated with C-3 of the aglycone at δ 81.6. All of these data are consistent with the structure 3 β -[(α -L-arabinopyranosyl)oxy]-11 α ,12 α -epoxy-13 β ,16 α ,23-trihydroxyoleanan-28-oic acid γ -lactone, which was assigned to **4**.

Compound **5** (C₃₅H₅₄O₉) was suggested to be a triterpene glycoside with a conjugated diene group by the UV [λ _{max} 252 nm (log ϵ 4.18)] and ¹³C NMR spectra [δ 136.4 (C) and 130.6 (C); δ 126.7 (CH) and 126.4 (CH)]. Enzymatic hydrolysis of **5** with naringinase gave an aglycone (**5a**; C₃₀H₄₆O₅) and L-arabinose. The spectroscopic properties of **5a** were closely related to those of the known triterpene 3 β ,23-dihydroxyoleana-11,13-dien-28-oic acid.¹⁶ The molecular formula of **5a** was higher by one oxygen atom than that of the known triterpene, and the ¹H and ¹³C NMR spectra of **5a** implied the presence of a C-16 α hydroxy group [δ _H 4.86 (t-like, J = 2.7 Hz; δ _C 70.4)] in addition to a C-3 β hydroxy group.

These data indicated that the structure of **5a** was $3\beta,16\alpha,23$ -trihydroxyoleana-11,13-dien-28-oic acid. In the HMBC spectrum of **5**, linkage of an α -L-arabinopyranosyl (Ara) group to C-3 of the aglycone was ascertained by a long-range correlation between the signals at δ_{H} 5.02 (d, $J = 7.2$ Hz, H-1 of Ara) and δ_{C} 81.7 (C-3 of the aglycone). The structure of **5** was assigned to be 3β -[(α -L-arabinopyranosyl)oxy]- $16\alpha,23$ -dihydroxyoleana-11,13-dien-28-oic acid.

Compound **6** ($\text{C}_{41}\text{H}_{68}\text{O}_{13}$) was shown to have the same diglycoside residue as **3**, but differed from **3** in terms of the aglycone moiety on the basis of the ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum of **6** displayed signals for six tertiary methyl groups (δ 1.45, 1.23, 1.05, 1.03, 1.02, 0.79), a secondary methyl group at δ 1.41 (d, $J = 6.2$ Hz), and an oxymethine proton geminally bearing the diglycoside of β -D-glucosyl-(1 \rightarrow 2)- α -L-arabinosyl. The deshielded methyl singlet signal at δ 1.45 (Me-30) showed long-range correlations with the signals for not only its attached carbon at δ 72.4 (C-20) but also a methine carbon attached to a methyl group at δ 39.9 (C-19) and a methylene carbon at δ 37.5 (C-21) in the HMBC spectrum of **6**. These data and NOE correlations between Me-30 and H-19 (δ 2.51)/H-21eq (δ 1.87)/H-21ax (δ 2.12), Me-29 (δ 1.41), and H-18 (δ 1.79), and between H-19 and H-21ax, together with the results of acid hydrolysis, allowed the aglycone moiety of **6** to be identified as 20α -hydroxyursan-28-oic acid (**6a**).¹⁷ Thus, the structure of **6** was assigned as 3β -[(O - β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl)oxy]- 20α -hydroxyursan-28-oic acid.

Compound **7** ($\text{C}_{41}\text{H}_{66}\text{O}_{12}$) was a triterpene diglycoside whose structure was closely related to that of **6** based on its ^1H and ^{13}C NMR spectra. However, the molecular formula of **7** was less than that of **6** by H_2O . The IR spectrum of **7** showed a prominent ester carbonyl absorption band at 1744 cm^{-1} . When the ^{13}C NMR spectrum of **7** was compared with that of **6**, the carbonyl carbon signal due to C-28 was shifted upfield by 2.8 ppm, whereas the C-20 oxymethine carbon appeared downfield by 11.5 ppm. These spectroscopic data suggested that **7** was a stereoisomer with regard to the C-20 hydroxy group, thus forming a six-membered lactone ring between C-20 and C-28. Enzymatic hydrolysis of **7** with naringinase furnished $3\beta,20\beta$ -dihydroxyursan-28-oic acid δ -lactone (**7a**),¹⁸ together with L-arabinose and D-glucose. Therefore, the structure of **7** was established as 3β -[(O - β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl)oxy]- 20β -hydroxyursan-28-oic acid δ -lactone.

Compounds **8** and **9** had the same molecular formula ($\text{C}_{41}\text{H}_{66}\text{O}_{14}$) by the HRESITOFMS and ^{13}C NMR with DEPT data and gave the common hydrolysates $3\beta,16\alpha,23$ -trihydroxyolean-12-en-28-oic acid (**8a**),¹² L-arabinose, and D-glucose. These data indicated that **8** and **9** were triterpene diglycosides related to each other in regard to the sugar linkage positions. Comparison of the NMR data of **8** with those of **3** and analyses of the ^1H - ^1H COSY, HMQC, and HMBC data of **8** showed that a β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl unit was linked to C-3 of the aglycone. On the other hand, the ^1H and ^{13}C NMR spectra of **9** gave evidence for the presence of a terminal α -L-arabinopyranosyl (Ara) unit and a terminal β -D-glucopyranosyl (Glc) unit in the molecule. The ^{13}C NMR shifts of C-3 at δ 82.0 and C-28 at δ 175.9 implied that sugar linkages were at both C-3 and C-28. In the HMBC spectrum of **9**, long-range correlations between H-1 of Ara at δ 5.00 (d, $J = 7.2$ Hz) and C-3 of the aglycone and between H-1 of Glc at δ 6.35 (d, $J = 8.1$ Hz) and C-28 of the aglycone were consistent with linkages of Ara at C-3 and Glc at C-28. Thus, the structures of **8** and **9** were determined to be 3β -[(O - β -D-glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl)oxy]- $16\alpha,23$ -dihydroxyolean-12-en-28-oic acid and 3β -[(α -L-arabinopyranosyl)oxy]- $16\alpha,23$ -dihydroxyolean-12-en-28-oic acid β -D-glucopyranosyl ester, respectively.

Compound **10** was isolated as an amorphous solid with a molecular formula of $\text{C}_{47}\text{H}_{76}\text{O}_{19}$, which was higher than that of **9** by $\text{C}_6\text{H}_{10}\text{O}_5$. The ^1H NMR spectrum of **10** contained signals for three anomeric protons, signals for six tertiary methyl groups, and

an olefinic proton signal at δ 5.58 characteristic of oleanolic acid. Enzymatic hydrolysis of **10** with naringinase gave **8a**, L-arabinose, and D-glucose. These data and comparison of the ^{13}C NMR spectrum of **10** with that of **9** suggested that **10** was related to **9** with an additional β -D-glucopyranosyl unit attached to the C-3 (δ 82.0) or C-28 (δ 176.0) sugar moiety. In the HMBC spectrum of **10**, H-1 of Glc' at δ 4.97 showed a long-range correlation with C-6 of the inner Glc at δ 69.3, of which H-1 of Glc at δ 6.21 exhibited a long-range correlation with C-28 of the aglycone at δ 176.0. An HMBC correlation was also observed between H-1 of an α -L-arabinopyranosyl residue at δ 4.99 and C-3 of the aglycone at δ 82.0. Thus the structure of **10** was assigned as 3β -[(α -L-arabinopyranosyl)oxy]- $16\alpha,23$ -dihydroxyolean-12-en-28-oic acid O - β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl ester.

Compounds **1**–**22** were evaluated for their cytotoxic activity against HL-60 cells. The triterpene monodesmosides based on oleanolic acid, **1**, and **11**–**16** showed cytotoxic activity with IC_{50} values of 4.8 ± 0.14 , 3.4 ± 0.17 , 12.5 ± 0.38 , 10.8 ± 0.24 , 15.9 ± 0.24 , 5.6 ± 0.11 , and $11.8 \pm 0.46\ \mu\text{g}/\text{mL}$, respectively. Etoposide, used as a positive control, had an IC_{50} value of $0.26 \pm 0.02\ \mu\text{g}/\text{mL}$. The other isolated triterpene glycosides were noncytotoxic to HL-60 cells at sample concentrations up to $20\ \mu\text{g}/\text{mL}$.

Experimental Section

General Experimental Procedures. Optical rotation data were measured with a DIP-360 (Jasco, Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded with a Jasco FT-IR 620 spectrophotometer. NMR spectra (500 MHz for ^1H NMR) were recorded with a DRX-500 spectrometer (Bruker, Karlsruhe, Germany), using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. HRESITOFMS data were obtained with an LCT mass spectrometer (Waters-Micromass, Manchester, UK). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), BW-300 silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography (CC). TLC was carried out on precoated Kieselgel 60 F₂₅₄ (0.25 mm thick, Merck, Darmstadt, Germany) and RP₁₈ F₂₅₄ S plates (0.25 mm thick, Merck), and the spots were visualized by spraying the plates with 10% H_2SO_4 and then heating. HPLC was performed with a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), RI-8010 (Tosoh) and Shodex OR-2 (Showa-Denko, Tokyo, Japan) detectors, and a Rheodyne injection port. A TSK gel ODS-100Z column (10 mm i.d. \times 250 mm, 5 μm , Tosoh) was employed for preparative HPLC. 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 plate (Iwaki Glass, Chiba, Japan); JCRB 0085 HL-60 cells (Human Science Research Resources Bank, Osaka, Japan); FBS (Bio-Whittaker, Walkersville, MD); RPMI 1640 medium, etoposide, and MTT (Sigma, St. Louis, MO); and penicillin G and streptomycin sulfate (Meiji-Seika, Tokyo, Japan). All other chemicals used were of biochemical reagent grade.

Plant Material. The underground parts of *C. thalictroides* were obtained from Richters, Ontario, Canada. A small amount of the sample is preserved in our laboratory (02-007-CT).

Extraction and Isolation. The underground parts of *C. thalictroides* (4.0 kg of dry weight) were extracted with hot MeOH (11 L). After removing the solvent, the MeOH extract (535 g) was passed through a Diaion HP-20 column and successively eluted with 30% MeOH, 60% MeOH, MeOH, EtOH, and EtOAc (each 10 L). CC of the MeOH-eluted fraction (80.0 g) on silica gel, eluted with a stepwise gradient mixture of CHCl_3 -MeOH- H_2O (90:10:1; 40:10:1; 20:10:1; 7:4:1) and finally with MeOH alone, provided 14 fractions (A–N). Fraction C was chromatographed on ODS silica gel eluted with MeOH- H_2O (6:1; 5:1; 4:1; 3:1) to yield **1** (8.3 mg), **8** (42.5 mg), **11** (8.6 mg), and **15** (50.2 mg). Fraction D was separated in an ODS silica gel column eluted with MeOH- H_2O (4:1) to give **12** (1.58 g). Fraction E was separated in an ODS silica gel column eluted with MeOH- H_2O (2:1) to give **4** (20.6 mg). Fraction F was chromatographed on silica gel eluted with CHCl_3 -MeOH- H_2O (90:10:1) and on ODS silica gel eluted with MeOH- H_2O (3:1) to afford **17** (802 mg). Fraction G was separated on a silica gel column eluted with CHCl_3 -MeOH- H_2O (90:10:1; 80:

Table 1. ^{13}C NMR Data for **1–4**, **4a**, **5**, **5a**, and **6–10** in Pyridine- d_5 at 300 K

position	1	2	3	4	4a	5	5a	6	7	8	9	10
1	38.1	38.2	38.4	38.4	38.5	38.4	38.3	38.9	39.0	38.7	38.9	38.8
2	25.2	25.3	25.6	25.7	27.1	26.1	27.5	26.7	26.6	25.9	26.1	26.0
3	81.4	81.3	81.8	81.6	72.5	81.7	73.0	88.9	88.8	82.1	82.0	82.0
4	55.5	55.6	43.6	43.5	43.0	43.6	43.0	39.6	39.6	43.4	43.5	43.4
5	47.8	47.9	47.3	47.0	47.7	47.4	48.2	55.8	58.8	47.9	47.8	47.6
6	20.4	20.5	17.4	17.3	17.6	18.2	18.5	18.5	18.3	18.1	18.2	18.2
7	32.4	32.8	31.0	31.1	31.1	32.4	32.4	35.0	34.2	33.1	33.2	33.0
8	39.9	40.1	41.6	41.5	41.4	41.8	41.7	41.5	40.7	39.8	42.1	40.1
9	47.7	47.1	51.2	50.4	50.3	54.2	54.1	50.3	50.7	47.2	47.3	47.2
10	36.0	36.1	36.3	36.3	36.5	36.6	36.8	36.9	37.0	36.9	37.0	36.9
11	23.5	23.7	52.7	52.7	52.7	126.7	126.4	21.9	21.1	23.8	23.9	23.8
12	122.1	122.0	57.2	57.2	57.2	126.4	126.7	29.7	25.3	122.3	122.8	122.6
13	144.8	145.1	87.6	88.6	88.6	136.4	136.4	40.8	43.2	145.0	144.4	144.5
14	42.1	42.1	40.8	40.5	40.5	42.0	42.0	43.0	41.3	42.0	40.2	42.0
15	28.1	36.0	27.0	37.2	37.1	33.3	33.2	29.9	27.6	36.0	36.2	35.8
16	23.7	74.6	21.5	72.3	72.2	70.4	70.4	36.1	28.0	74.6	74.4	74.2
17	46.5	48.8	44.0	48.1	48.1	54.2	54.2	51.4	42.2	48.8	49.1	49.1
18	42.0	41.4	49.7	50.5	50.4	130.6	130.6	47.9	48.3	41.3	41.3	41.2
19	46.3	47.2	37.9	39.0	39.0	41.3	41.2	39.9	42.3	47.1	47.1	47.1
20	30.9	31.0	31.4	31.5	31.4	32.3	32.3	72.4	83.9	30.9	30.8	30.7
21	34.1	36.1	34.3	36.0	35.9	37.2	37.1	37.5	27.2	36.1	35.9	36.1
22	33.1	32.8	27.6	28.1	28.0	29.4	29.4	33.9	32.2	32.7	32.2	32.0
23	207.0	206.9	64.4	64.1	66.7	64.2	67.3	28.1	28.1	64.7	64.5	64.4
24	10.3	10.3	12.9	13.0	12.5	13.0	12.5	16.4	16.5	13.4	13.6	13.5
25	15.5	15.6	17.7	17.7	17.5	18.9	18.7	16.6	16.4	16.1	16.3	16.3
26	17.2	17.4	20.3	20.0	19.9	17.2	17.2	16.7	15.8	17.4	17.6	17.6
27	26.0	27.1	18.8	18.5	18.5	21.4	21.4	15.1	14.3	27.1	27.1	27.1
28	180.1	179.9	179.0	178.6	178.7	179.0	179.0	179.4	176.6	179.9	175.9	176.0
29	33.2	33.3	33.0	33.2	33.1	32.5	24.8	19.1	18.6	33.2	33.2	33.0
30	23.6	24.7	23.4	24.3	24.2	24.8	32.5	30.8	24.1	24.7	24.6	24.6
Ara 1	105.4	105.4	104.0	106.6		106.6		104.9	104.8	103.9	106.7	106.5
2	72.5	72.5	81.3	73.1		73.1		81.1	81.0	81.1	73.1	73.1
3	74.4	74.4	73.6	74.7		74.7		73.4	73.4	73.5	74.7	74.6
4	69.3	69.3	68.3	69.6		69.6		68.3	68.3	68.3	69.6	69.5
5	66.8	66.8	65.0	66.9		66.9		65.0	64.9	65.0	67.0	66.8
Glc 1			105.9					106.1	105.9	105.7	95.9	95.7
2			76.2					76.4	76.4	76.2	74.2	73.8
3			78.2					78.2	78.2	78.2	78.9	78.2
4			71.4					71.6	71.5	71.2	71.1	70.7
5			78.3					78.2	78.2	78.1	79.4	77.8
6			62.5					62.6	62.5	62.3	62.2	69.3
Glc 1'												105.1
2'												75.0
3'												78.5
4'												71.4
5'												78.3
6'												62.4

10:1; 70:10:1; 50:10:1) and EtOAc–MeOH–H₂O (130:10:1; 50:10:1) and on an ODS silica gel column eluted with MeOH–H₂O (10:1; 7:1; 4:1) and MeCN–H₂O (1:1) to give **2** (19.2 mg), **5** (43.2 mg), and **7** (10.0 mg). Fraction H was chromatographed on silica gel eluted with CHCl₃–MeOH–H₂O (70:10:1) and EtOAc–MeOH–H₂O (130:10:1; 50:10:1) and on ODS silica gel eluted with MeOH–H₂O (10:1; 6:1; 4:1) and MeCN–H₂O (6:7) to afford **13** (51.3 mg). Fraction I was chromatographed on silica gel eluted with CHCl₃–MeOH–H₂O (50:10:1) and EtOAc–MeOH–H₂O (70:10:1; 50:10:1) and on ODS silica gel eluted with MeOH–H₂O (3:1; 2:1) to afford **3** (13.8 mg), **14** (164 mg), and **16** (30.3 mg). Fraction J was separated by HPLC using MeCN–H₂O (3:4) to give **6** (8.5 mg). Fraction L was chromatographed on silica gel eluted with CHCl₃–MeOH–H₂O (90:10:1; 7:4:1) and on ODS silica gel eluted with MeCN–H₂O (3:5) to afford **9** (16.0 mg). Fraction M was subjected to silica gel CC eluted with CHCl₃–MeOH–H₂O (20:10:1) and ODS silica gel CC eluted with MeOH–H₂O (8:5) and MeCN–H₂O (6:13) and finally purified by HPLC using MeCN–H₂O (2:1) to yield **10** (7.5 mg), **18** (76.9 mg), **19** (314 mg), **20** (42.0 mg), and **21** (2.61 g).

Compound 1: amorphous solid; $[\alpha]_{\text{D}}^{23} +50.0$ (c 0.10, MeOH); IR (film) ν_{max} 3390 (OH), 2927 and 2856 (CH), 1717 and 1692 (C=O) cm^{-1} ; ^1H NMR (C₅D₅N, 500 MHz) δ 9.73 (1H, s, H-23), 5.45 (1H, t-like, $J = 3.0$ Hz, H-12), 4.11 (1H, dd, $J = 12.6, 4.2$ Hz, H-3), 1.27 \times 2 (each 3H, s, Me-24 and Me-27), 0.99 (3H, s, Me-30), 0.94 (3H, s, Me-29), 0.93 (3H, s, Me-26), 0.82 (3H, s, Me-25), signals for the sugar moiety, see Table 2; ^{13}C NMR, see Table 1; HRESITOFMS m/z 603.3943 [M + H]⁺ (calcd. for C₃₅H₅₅O₈, 603.3897).

Reduction of 1. NaBH₄ (3.1 mg) was added to a solution of **1** (2.4 mg) in EtOH (1 mL), and then the mixture was stirred at room

temperature for 5 h. Acetone (1 mL) was added to the reaction mixture, and the solvent was evaporated *in vacuo* to give a residue. After the residue was diluted with H₂O (3 mL), washed successively with EtOAc (3 mL \times 3), and concentrated under reduced pressure, it was purified by CC on silica gel using CHCl₃–MeOH (9:1) to give **12** (2.4 mg).

Compound 2: amorphous solid; $[\alpha]_{\text{D}}^{22} +84.0$ (c 0.10, MeOH); IR (film) ν_{max} 3366 (OH), 2926 and 2855 (CH), 1726 and 1674 (C=O) cm^{-1} ; ^1H NMR (C₅D₅N, 500 MHz) δ 9.70 (1H, s, H-23), 5.62 (1H, t-like, $J = 3.4$ Hz, H-12) 5.22 (1H, br s, H-16), 4.65 (1H, d, $J = 7.0$ Hz, Ara-1), 4.14 (1H, dd, $J = 11.7, 4.5$ Hz, H-3), 3.59 (1H, dd, $J = 14.4, 4.2$ Hz, H-18), 2.30 (1H, dd, $J = 14.6, 3.2$ Hz, H-15a), 1.82 (3H, s, Me-27), 1.68 (1H, dd, $J = 14.6, 2.6$ Hz, H-15b), 1.27 (3H, s, Me-24), 1.17 (3H, s, Me-30), 1.05 (3H, s, Me-29), 0.97 (3H, s, Me-26), 0.87 (3H, s, Me-25); ^{13}C NMR, see Table 1; HRESITOFMS m/z 641.3714 [M + Na]⁺ (calcd. for C₃₅H₅₄O₉Na, 641.3666).

Reduction of 2. Compound **2** (5.1 mg) in MeOH (1 mL) was treated with NaBH₄ (10.0 mg), and a workup similar to that for **1** gave **17** (8.4 mg).

Compound 3: amorphous solid; $[\alpha]_{\text{D}}^{23} +34.0$ (c 0.10, MeOH); IR (film) ν_{max} 3388 (OH), 2929 and 2878 (CH), 1774 (C=O) cm^{-1} ; ^1H NMR (C₅D₅N, 500 MHz) δ 4.24 (1H, d, $J = 10.9$ Hz, H-23a), 4.14 (1H, dd, $J = 11.9, 4.7$ Hz, H-3), 3.73 (1H, d, $J = 10.9$ Hz, H-23b), 3.23 (1H, br d, $J = 3.8$ Hz, H-12), 3.10 (1H, dd, $J = 3.8, 1.3$ Hz, H-11), 2.50 (1H, dd, $J = 13.6, 3.0$ Hz, H-18), 1.15 (3H, s, Me-26), 1.13 (3H, s, Me-27), 1.01 (3H, s, Me-24), 0.99 (3H, s, Me-25), 0.86 (3H, s, Me-29), 0.78 (3H, s, Me-30), signals for the sugar moiety, see Table 2; ^{13}C NMR, see Table 1; HRESITOFMS m/z 781.4406 [M + H]⁺ (calcd. for C₄₁H₆₅O₁₄, 781.4374).

Table 2. ¹H NMR Data for the Sugar Moieties of **1**, **3**, **9**, and **10** in Pyridine-*d*₅ at 300 K

1			3			9			10		
position	¹ H	<i>J</i> (Hz)	position	¹ H	<i>J</i> (Hz)	position	¹ H	<i>J</i> (Hz)	position	¹ H	<i>J</i> (Hz)
Ara 1	4.65 d	7.0	Ara 1	5.15 d	6.1	Ara 1	5.00 d	7.2	Ara 1	4.99 d	7.6
2	4.30 dd	8.9,7.0	2	4.57 dd	7.5,6.1	2	4.43 dd	9.4,7.2	2	4.41 dd	8.9,7.6
3	4.11 dd	8.9,3.5	3	4.25 br d	7.3	3	4.06 dd	9.4,3.4	3	4.10 dd	8.9,3.3
4	4.29 br s		4	4.30 m		4	4.25 br s		4	4.25 br s	
5	4.31 br d	12.9	5	4.28 dd	11.4,1.7	5	4.29 br d	11.8	5	4.26 br d	12.7
	3.76 dd	12.9,2.4		3.69 dd	11.4,1.7		3.72 br d	11.8		3.72 dd	12.7,1.9
			Glc 1	5.18 d	7.7	Glc 1	6.35 d	8.1	Glc 1	6.21 d	8.3
			2	4.08 dd	8.5,7.7	2	4.16 dd	9.3,8.1	2	4.05 dd	8.6,8.3
			3	4.17 dd	9.0,8.5	3	4.27 dd	9.3,9.1	3	4.19 dd	9.5,8.6
			4	4.22 dd	9.0,8.5	4	4.33 dd	9.1,9.1	4	4.29 dd	9.8,9.5
			5	3.82 ddd	8.5, 4.5, 2.6	5	4.03 ddd	9.1, 4.2, 2.7	5	4.04	overlapping
			6	4.47 dd	11.7,2.6	6	4.46 dd	12.4,2.7	6	4.67 br d	9.7
				4.38 dd	11.7,4.5		4.40 dd	12.4,4.2		4.30 br d	11.1
									Glc 1'	4.97 d	7.6
									2'	3.96 dd	8.4,7.6
									3'	4.20	overlapping
									4'	4.18	overlapping
									5'	3.85 m	
									6'	4.44 dd	12.2,2.3
										4.32 dd	12.2,5.0

Table 3. Cytotoxic Activity of **1–22** against HL-60 Cells

compd	IC ₅₀ (μg/mL) ^a	compd	IC ₅₀ (μg/mL) ^a
1	4.8 ± 0.14	12	12.5 ± 0.38
2	>20	13	10.8 ± 0.24
3	>20	14	15.9 ± 0.24
4	>20	15	5.6 ± 0.11
5	>20	16	11.8 ± 0.46
6	>20	17	>20
7	>20	18	>20
8	>20	19	>20
9	>20	20	>20
10	>20	21	>20
11	3.4 ± 0.17	22	>20
etoposide	0.3 ± 0.02		

^a Data are presented as the mean value ± SEM of three experiments performed in triplicate.

Enzymatic Hydrolysis of 3. Compound **3** (9.6 mg) was treated with naringinase (Sigma, EC 232-96-4) (30.2 mg) in an HOAc–KOAc buffer (pH 4.3, 10 mL) at room temperature for 72 h. The reaction mixture was passed through a Sep-Pak C18 cartridge and a Toyopak IC-SP M cartridge, eluted with H₂O followed by MeOH. The MeOH-eluted fraction was chromatographed on silica gel eluted with CHCl₃–MeOH (19:1) to yield **3a** (4.1 mg). The H₂O-eluted fraction (0.8 mg) was analyzed by HPLC under the following conditions: column, Capcell Pak NH₂ UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Japan); solvent, MeCN–H₂O (85:15); detection, RI and OR; flow rate, 1.0 mL/min. L-Arabinose and D-glucose were identified by comparison of their retention times and specific rotations values with those of authentic samples.

Compound 4: amorphous solid; [α]_D²³ +60.0 (c 0.10, MeOH); IR (film) ν_{max} 3376 (OH), 2949 (CH), 1765 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 4.98 (1H, d, *J* = 7.0 Hz, Ara-1), 4.46 (1H, br d, *J* = 4.8 Hz, H-16), 4.30 (1H, d, *J* = 10.9 Hz, H-23a), 4.26 (1H, dd, *J* = 11.1, 5.1 Hz, H-3), 3.66 (1H, d, *J* = 10.9 Hz, H-23b), 3.35 (1H, d, *J* = 3.8 Hz, H-12), 3.18 (1H, br d, *J* = 3.8 Hz, H-11), 2.72 (1H, dd, *J* = 14.1, 2.6 Hz, H-18), 2.06 (1H, dd, *J* = 14.5, 4.8 Hz, H-15ax), 1.79 (3H, s, Me-27), 1.53 (1H, br d, *J* = 14.5 Hz, H-15eq), 1.16 (3H, s, Me-26), 1.03 (3H, s, Me-25), 0.96 (3H, s, Me-29), 0.92 (3H, s, Me-30), 0.91 (3H, s, Me-24); ¹³C NMR, see Table 1; HRESITOFMS *m/z* 657.3610 [M + Na]⁺ (calcd. for C₃₅H₅₄O₁₀Na, 657.3615).

Enzymatic Hydrolysis of 4. A solution of **4** (11.1 mg) with naringinase (50.2 mg) was subjected to enzymatic hydrolysis as described for **3** to give **4a** (5.7 mg) and a sugar fraction (0.7 mg). HPLC analysis of this sugar fraction under the same conditions as those used for **3** showed the presence of L-arabinose and D-glucose.

Compound 4: amorphous solid; [α]_D²³ +28.0 (c 0.10, CHCl₃); IR (film) ν_{max} 3397 (OH), 2947 and 2866 (CH), 1759 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 4.48 (1H, br d, *J* = 5.0 Hz, H-16), 4.22 (1H, dd, *J* = 11.4, 4.8 Hz, H-3), 4.16 (1H, d, *J* = 10.7 Hz, H-23a), 3.63 (1H, d, *J* = 10.7 Hz, H-23b), 3.35 (1H, d, *J* = 3.8 Hz, H-12),

3.19 (1H, dd, *J* = 3.6, 1.5 Hz, H-11), 2.70 (1H, dd, *J* = 14.1, 2.8 Hz, H-18), 2.04 (1H, dd, *J* = 14.6, 5.0 Hz, H-15ax), 1.77 (3H, s, Me-27), 1.57 (1H, br d, *J* = 14.6 Hz, H-15eq), 1.15 (3H, s, Me-26), 1.04 (3H, s, Me-25), 1.00 (3H, s, Me-24), 0.93 (3H, s, Me-29), 0.90 (3H, s, Me-30); ¹³C NMR, see Table 1; HRESITOFMS *m/z* 503.3359 [M + H]⁺ (calcd. for C₃₀H₄₇O₆, 503.3373).

Compound 5: amorphous solid; [α]_D²³ –22.0 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 252 (4.18) nm; IR (film) ν_{max} 3388 (OH), 2940 and 2858 (CH), 1698 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 6.82 (1H, dd, *J* = 10.8, 2.7 Hz, H-12), 5.80 (1H, br d, *J* = 10.8 Hz, H-11), 5.02 (1H, d, *J* = 7.2 Hz, Ara-1), 4.85 (1H, t-like, *J* = 3.2 Hz, H-16), 4.33 (1H, dd, *J* = 11.9, 4.9 Hz, H-3), 4.32 (1H, d, *J* = 10.8 Hz, H-23a), 3.70 (1H, d, *J* = 10.8 Hz, H-23b), 2.30 (1H, br s, H-9), 1.69 (3H, s, Me-27), 1.15 (3H, s, Me-26), 1.04 × 2 (each 3H, s, Me-25 and Me-30), 0.98 (3H, s, Me-29), 0.91 (3H, s, Me-24); ¹³C NMR, see Table 1; HRESITOFMS *m/z* 641.3694 [M + Na]⁺ (calcd. for C₃₅H₅₄O₉Na, 641.3666).

Enzymatic Hydrolysis of 5. A solution of **5** (12.2 mg) with naringinase (56.4 mg) was subjected to enzymatic hydrolysis as described for **3** to yield **5a** (5.4 mg) and a sugar fraction (0.9 mg). HPLC analysis of this sugar fraction under the same conditions as in the case of **3** showed the presence of L-arabinose.

Compound 5: amorphous solid; [α]_D²² –80.0 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 253 (4.12) nm; IR (film) ν_{max} 3388 (OH), 2930 and 2858 (CH), 1696 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 6.83 (1H, dd, *J* = 10.6, 2.6 Hz, H-11), 5.82 (1H, d, *J* = 10.6 Hz, H-12), 4.86 (1H, t-like, *J* = 2.7 Hz, H-16), 4.28 (1H, dd, *J* = 11.2, 5.2 Hz, H-3), 4.19 (1H, d, *J* = 10.5 Hz, H-23a), 3.71 (1H, d, *J* = 10.5 Hz, H-23b), 2.87 (1H, d, *J* = 14.5 Hz, H-19eq), 2.75 (1H, ddd, *J* = 14.0, 14.0, 3.8 Hz H-22ax), 2.55 (1H, ddd, *J* = 14.0, 3.8, 3.1 Hz H-22eq), 2.37 (1H, d, *J* = 14.5 Hz, H-19ax), 2.29 (1H, br s, H-9), 2.22 (1H, dd, *J* = 14.0, 2.7 Hz, H-15ax), 1.80 (1H, dd, *J* = 14.0, 2.7 Hz, H-15eq), 1.69 (3H, s, Me-27), 1.61 (1H, br d, *J* = 12.0 Hz, H-5), 1.16 (3H, s, Me-26), 1.07 (3H, s, Me-25), 1.03 × 2 (each 3H, s, Me-24 and Me-29), 0.97 (3H, s, Me-30); ¹³C NMR, see Table 1; HRESITOFMS *m/z* 487.3462 [M + H]⁺ (calcd. for C₃₀H₄₇O₅, 487.3424).

Compound 6: amorphous solid; [α]_D²⁶ –10.7 (c 0.31, MeOH); IR (film) ν_{max} 3404 (OH), 2939 (CH), 1642 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 5.20 (1H, d, *J* = 7.7 Hz, Glc-1), 5.00 (1H, d, *J* = 5.7 Hz, Ara-1), 3.26 (1H, dd, *J* = 11.6, 4.0 Hz, H-3), 2.39 (1H, dd, *J* = 12.2, 3.0 Hz, H-16a), 1.59 (1H, dd, *J* = 13.9, 13.0, 3.1 Hz, H-16), 1.37 (1H, m, H-12), 2.51 (1H, m, H-19), 2.36 (1H, ddd, *J* = 12.9, 3.0, 3.0 Hz, H-22ax), 2.12 (1H, dt, *J* = 13.9, 2.7 Hz, H-21ax), 2.04 (1H, ddd, *J* = 12.6, 2.7, 2.7 Hz, H-22eq), 1.87 (1H, m, H-21eq), 1.79 (1H, t, *J* = 10.4 Hz, H-18), 1.45 (3H, s, Me-30), 1.41 (3H, d, *J* = 6.2 Hz, Me-29), 1.23 (3H, s, Me-23), 1.05 (3H, s, Me-26), 1.03 (3H, s, Me-24), 1.02 (3H, s, Me-27), 0.79 (3H, s, Me-25); ¹³C NMR, see Table 1; HRESITOFMS *m/z* 791.4556 [M + Na]⁺ (calcd. for C₄₁H₆₈O₁₃Na, 791.4558).

Acid Hydrolysis of 6. A solution of **6** (5.8 mg) in 1 M HCl (dioxane–H₂O, 1:1; 2 mL) was heated at 95 °C for 2 h under an Ar

atmosphere. After cooling, the reaction mixture was diluted with H₂O (6 mL) and extracted with CHCl₃ (5 mL × 3). TLC analysis of the CHCl₃-soluble fraction showed that the labile aglycone decomposed under acidic conditions. The H₂O-soluble fraction was neutralized by passing through an Amberlite IRA-96SB (Organo, Tokyo, Japan) column and then passed through a Sep-Pak C18 cartridge, using 40% MeOH to give a sugar fraction (2.3 mg). The sugar fraction analyzed by HPLC under the same conditions as in the case of **3** showed L-arabinose and D-glucose.

Compound 7: amorphous solid; $[\alpha]_D^{21} +4.0$ (*c* 0.10, MeOH); IR (film) ν_{\max} 3376 (OH), 2925, 2871, and 2855 (CH), 1744 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 5.19 (1H, d, *J* = 7.7 Hz, Ara-1), 4.98 (1H, d, *J* = 5.7 Hz, Glc-1), 3.23 (1H, dd, *J* = 11.8, 4.5 Hz, H-3), 1.26 (3H, s, Me-30), 1.24 (1H, t-like, *J* = 12.9 Hz, H-13), 1.22 (3H, s, Me-23), 1.04 (1H, dd, *J* = 11.9, 5.5 Hz, H-18), 1.03 (3H, s, Me-24), 0.89 (3H, s, Me-27), 0.88 (3H, d, *J* = 5.9 Hz, Me-29), 0.87 (3H, s, Me-26), 0.76 (3H, s, Me-25); ¹³C NMR, see Table 1; HRESITOFMS *m/z* 751.4648 [M + H]⁺ (calcd. for C₄₁H₆₇O₁₂, 751.4633).

Enzymatic Hydrolysis of 7. A solution of **7** (4.1 mg) with naringinase (35.2 mg) was subjected to enzymatic hydrolysis as described for **3** to yield **7a** (2.6 mg) and a sugar fraction (0.6 mg). HPLC analysis showed the presence of L-arabinose and D-glucose.

Compound 8: amorphous solid; $[\alpha]_D^{25} +15.6$ (*c* 0.10, MeOH); IR (film) ν_{\max} 3385 (OH), 2928 (CH), 1696 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 5.61 (1H, br s, H-12), 5.21 (1H, br s, H-16), 5.17 (1H, d, *J* = 7.8 Hz, Glc-1), 5.16 (1H, d, *J* = 5.9 Hz, Ara-1), 4.20 (1H, d, *J* = 11.0 Hz, H-23a), 4.15 (1H, dd, *J* = 11.7, 4.5 Hz, H-3), 3.78 (1H, d, *J* = 11.0 Hz, H-23b), 3.57 (1H, dd, *J* = 14.0, 4.0 Hz, H-18), 1.75 (3H, s, Me-27), 1.14 (3H, s, Me-30), 1.02 (3H, s, Me-26), 1.01 (3H, s, Me-29), 0.99 (3H, s, Me-24), 0.93 (3H, s, Me-25); ¹³C NMR, see Table 1; HRESITOFMS *m/z* 783.4514 [M + H]⁺ (calcd. for C₄₁H₆₇O₁₄, 783.4531).

Enzymatic Hydrolysis of 8. A solution of **8** (10.5 mg) with naringinase (70.0 mg) was subjected to enzymatic hydrolysis as described for **3** to yield **8a** (0.7 mg) and a sugar fraction (0.7 mg). HPLC analysis of this sugar fraction showed the presence of L-arabinose and D-glucose.

Compound 9: amorphous solid; $[\alpha]_D^{26} +2.0$ (*c* 0.86, MeOH); IR (film) ν_{\max} 3428 (OH), 2942 (CH), 1643 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 5.62 (1H, br s, *W*_{1/2} = 12.2 Hz, H-12), 5.29 (1H, br s, *W*_{1/2} = 16.2 Hz, H-16), 4.30 (1H, overlapping, H-3), 4.29 (1H, d, *J* = 12.8 Hz, H-23a), 3.69 (1H, d, *J* = 12.8 Hz, H-23b), 3.53 (1H, dd, *J* = 14.2, 4.2 Hz, H-18), 1.78 (3H, s, Me-27), 1.17 (3H, s, Me-26), 1.04 (3H, s, Me-30), 0.98 (3H, s, Me-29), 1.01 (3H, s, Me-25), 0.94 (3H, s, Me-24), signals for the sugar moieties, see Table 2; ¹³C NMR, see Table 1; HRESITOFMS *m/z*: 783.4579 [M + H]⁺ (calcd for C₄₁H₆₇O₁₄, 783.4531).

Acid Hydrolysis of 9. A solution of **9** (5.0 mg) was subjected to acid hydrolysis as described for **6** to give **8a** (3.6 mg) and a sugar fraction (2.2 mg). HPLC analysis of the sugar fraction under the same conditions as those used for **3** showed the presence of L-arabinose and D-glucose.

Compound 10: amorphous solid; $[\alpha]_D^{26} -46.0$ (*c* 0.10, MeOH); IR (film) ν_{\max} 3384 (OH), 2924 and 2857 (CH), 1733 and 1645 (C=O) cm⁻¹; ¹H NMR (C₅D₅N, 500 MHz) δ 5.58 (1H, br s, H-12), 5.27 (1H, br s, H-16), 4.26 (1H, d, *J* = 11.0 Hz, H-23a), 4.22 (1H, br d, *J* = 5.9 Hz, H-3), 3.65 (1H, d, *J* = 11.0 Hz, H-23b), 3.47 (1H, dd, *J* = 14.3, 4.3 Hz, H-18), 1.74 (3H, s, Me-27), 1.13 (3H, s, Me-26), 1.00 (3H, s, Me-30), 0.99 (3H, s, Me-25), 0.92 (3H, s, Me-29), 0.91 (3H, s, Me-24), signals for the sugar moieties, see Table 2; ¹³C NMR, see Table 1; HRESITOFMS *m/z* 967.4857 [M + Na]⁺ (calcd for C₄₇H₇₆O₁₉Na, 967.4879).

Enzymatic Hydrolysis of 10. A solution of **10** (5.0 mg) with naringinase (20.0 mg) was subjected to enzymatic hydrolysis as

described for **3** to yield **8a** (4.4 mg) and a sugar fraction (0.2 mg). HPLC analysis of this sugar fraction under the same conditions as in the case of **3** showed the presence of L-arabinose and D-glucose.

Cytotoxic Assay. HL-60 cells were maintained in an RPMI 1640 medium containing 10% FBS supplemented with L-glutamine, 100 units/mL of penicillin G, and 100 μg/mL of streptomycin sulfate. The leukemia cells were washed and resuspended in this medium to 4 × 10⁴ cells/mL, and 196 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in 5% CO₂/air for 24 h at 37 °C. After incubation, 4 μL of an EtOH-H₂O (1:1) solution containing the sample was added to give the final concentrations of 0.1–20 μg/mL, and 4 μL of EtOH-H₂O (1:1) was added into the control wells. The cells were further incubated for 72 h in the presence of each agent, and then the cell growth was evaluated by a modified MTT reduction assay. Briefly, after terminating the cell culture, 10 μL of 5 mg/mL of MTT in PBS was added to every well, and the plate was reincubated in 5% CO₂/air for 4 h at 37 °C. The plate was then centrifuged at 1500g for 5 min to precipitate the cells and MTT formazan. An aliquot of 150 μL of the 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 microshaker for 10 min and then read on a microplate reader at 550 nm.

Acknowledgment. We are grateful to Dr. Y. Shida and Mr. H. Fukaya, Tokyo University of Pharmacy and Life Sciences, for the mass spectra and elemental analyses.

Supporting Information Available: ¹H and ¹³C NMR data for **1–4**, **4a**, **5**, **5a**, and **6–10**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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NP900164B