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, Hachiouji, Tokyo 192-0392, Japan, and Faculty of Pharmaceutical Sciences, Aomori University, 2-3-1, Koubata, Aomori, Aomori 030-0943, Japan

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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 IC_{50} 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 magnoflorine[4]), steroidal glycosides (caulosaponin and caulophyllosaponin[5]), and triterpene glycosides[6] 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 octadecylsilanized (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-glucopyranosy1-(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-glucopyranosy1-(1→6)-β-D-glucopyranosyl ester (**18**),[13] 3β-[(α-L-arabinopyranosyl)oxy]-16α,23-dihydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosy1-(1→6)-β-D-glucopyranosy1 ester (**19**),[6] 3β-[(*O*-β-D-glucopyranosy1-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)- *O*-β-D-glucopyranosy1-(1→6)-β-D-glucopyranosy1 ester (**20**),[14] 3β-[(*O*-β-D-glucopyranosy1-(1→2)-α-L-arabinopyranosyl)oxy]-23-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosy1-(1→6)-β-D-glucopyranosy1 ester (**21**),[6] and 3β,23-dihydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosy1-(1→6)-β-D-glucopyranosy1 ester (**22**),[15] respectively.

Compound **1** was obtained as an amorphous solid, $[\alpha]_D$ +50.0 in MeOH, with a molecular formula of $C_{35}H_{54}O_8$, which was assigned on the basis of the HRESITOFMS (*m*/*z* 603.3943 $[M + H]^+$, calcd 603.3897) and ^{13}C 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 ^{13}C 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 ^{13}C NMR spectra of **1** instead of signals for the hydroxymethyl group of H_2-23/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 $NaBH_4$ 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 $C_{35}H_{54}O_9$ as determined by HRESITOFMS (*m*/*z* 641.3714 $[M + Na]^+$) and ^{13}C NMR data. Although the ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**, compound **2** had one additional oxygen atom. When the ^{13}C 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 H_2-15 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 $NaBH_4$ 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 $C_{41}H_{64}O_{14}$ from the HRESITOFMS data, showing an $[M + H]^+$ ion at *m*/*z* 781.4406, and the ^{13}C 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: matsuoy@toyaku.ac.jp; mimakiy@toyaku.ac.jp.
† Tokyo University of Pharmacy and Life Sciences.
‡ Aomori University.

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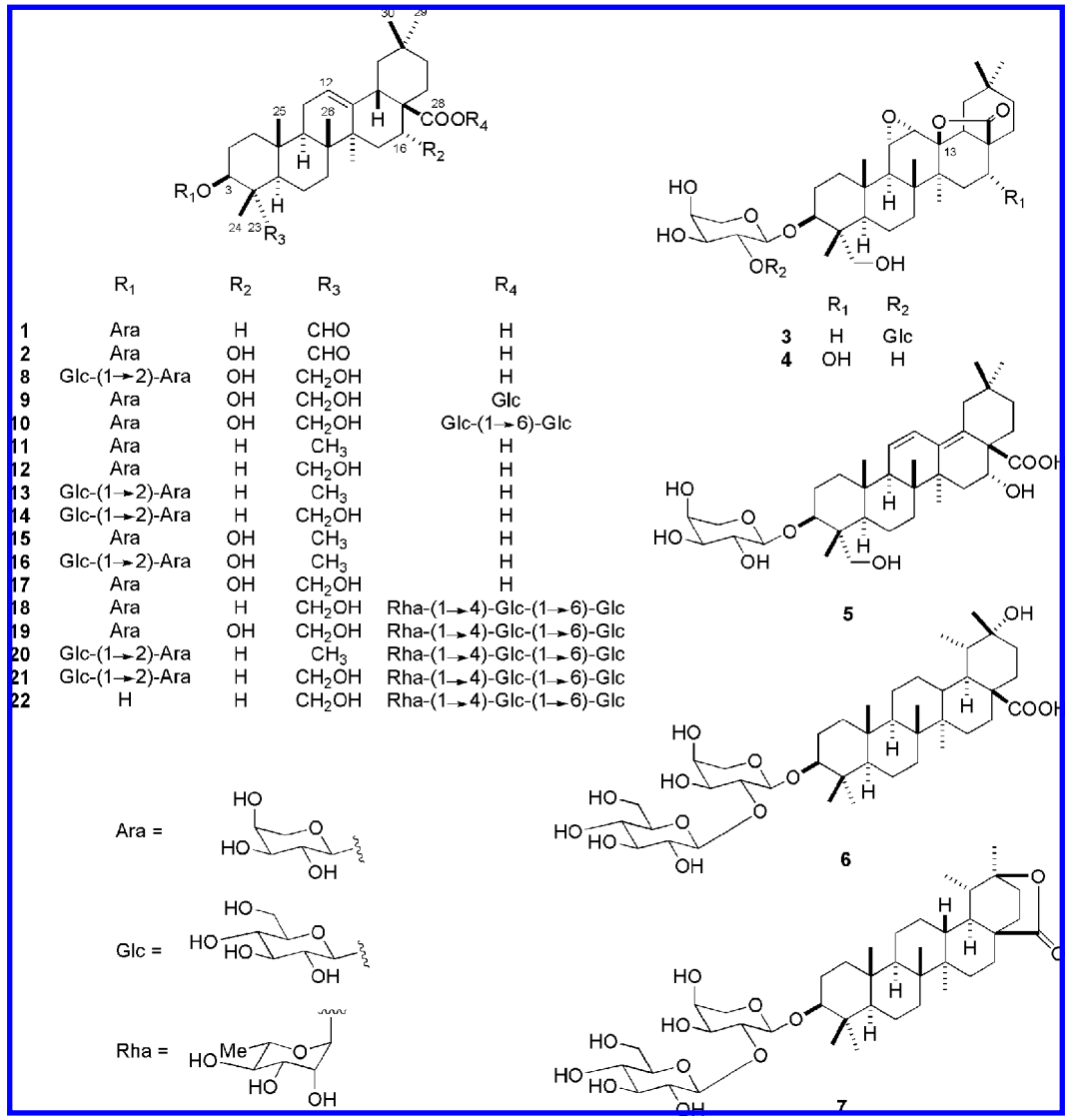
Chart 1



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, H_2-23), 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 ^{13}C 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 11α,12α-epoxy-3β,13β,23-dihydroxyoleanan-28-oic acid γ-lactone (**3a**; $C_{30}H_{46}O_5$),[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 3β-[(*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-11α,12α-epoxy-13β,23-dihydroxyoleanan-28-oic acid γ-lactone.

Compound **4** had the molecular formula $C_{35}H_{54}O_{10}$ on the basis of HRESITOFMS (*m*/*z* 657.3610 $[M + Na]^+$) and ^{13}C NMR data. Enzymatic hydrolysis of **4** with naringinase gave an aglycone (**4a**; $C_{30}H_{46}O_6$) and L-arabinose. Analyses of the ^1H and ^{13}C 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 H_2-15 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-16α hydroxy derivative of **3a**. The structure of **4a** was thus elucidated as 11α,12α-epoxy-3β,13β,16α,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 3β-[(α-L-arabinopyranosyl)oxy]-11α,12α-epoxy-13β,16α,23-trihydroxyoleanan-28-oic acid γ-lactone, which was assigned to **4**.

Compound **5** ($C_{35}H_{54}O_9$) was suggested to be a triterpene glycoside with a conjugated diene group by the UV [λ_{max} 252 nm (log ϵ 4.18)] and ^{13}C 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**; $C_{30}H_{46}O_5$) and L-arabinose. The spectroscopic properties of **5a** were closely related to those of the known triterpene 3β,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 ^{13}C NMR spectra of **5a** implied the presence of a C-16α hydroxy group [δ_H 4.86 (t-like, J = 2.7 Hz; δ_C 70.4)] in addition to a C-3β hydroxy group.

These data indicated that the structure of **5a** was 3β,16α,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]-16α,23-dihydroxyoleana-11,13-dien-28-oic acid.

Compound **6** ($C_{41}H_{68}O_{13}$) 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 ^{1}H and ^{13}C NMR spectra. The ^{1}H 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.

Compund **7** ($C_{41}H_{66}O_{12}$) was a triterpene diglycoside whose structure was closely related to that of **6** based on its ^{1}H 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 3β,20β-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 ($C_{41}H_{66}O_{14}$) by the HRESITOFMS and ^{13}C NMR with DEPT data and gave the common hydrolysates 3β,16α,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 ^{1}H$-^1$H 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 ^{1}H 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]-16α,23-dihydroxyolean-12-en-28-oic acid and 3β-[(α-L-arabinopyranosyl)oxy]-16α,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 $C_{47}H_{76}O_{19}$, which was higher than that of **9** by $C_6H_{10}O_5$. The ^{1}H 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]-16α,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 $\pm$ 0.14, 3.4 $\pm$ 0.17, 12.5 $\pm$ 0.38, 10.8 $\pm$ 0.24, 15.9 $\pm$ 0.24, 5.6 $\pm$ 0.11, and 11.8 $\pm$ 0.46 μg/mL, respectively. Etoposide, used as a positive control, had an IC_{50} value of 0.26 $\pm$ 0.02 μg/mL. The other isolated triterpene glycosides were noncytotoxic to HL-60 cells at sample concentrations up to 20 μg/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 ^{1}H 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 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP_{18} F_{254} 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−H_2O (130:10:1; 50:10:1) and on an ODS silica gel column eluted with MeOH−H_2O (10:1; 7:1; 4:1) and MeCN−H_2O (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 $CHCl_3$−MeOH−H_2O (70:10:1) and EtOAc−MeOH−H_2O (130:10:1; 50:10:1) and on ODS silica gel eluted with MeOH−H_2O (10:1; 6:1; 4:1) and MeCN−H_2O (6:7) to afford **13** (51.3 mg). Fraction I was chromatographed on silica gel eluted with $CHCl_3$−MeOH−H_2O (50:10:1) and EtOAc−MeOH−H_2O (70:10:1; 50:10:1) and on ODS silica gel eluted with MeOH−H_2O (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−H_2O (3:4) to give **6** (8.5 mg). Fraction L was chromatographed on silica gel eluted with $CHCl_3$−MeOH−H_2O (90:10:1; 7:4:1) and on ODS silica gel eluted with MeCN−H_2O (3:5) to afford **9** (16.0 mg). Fraction M was subjected to silica gel CC eluted with $CHCl_3$−MeOH−H_2O (20:10:1) and ODS silica gel CC eluted with MeOH−H_2O (8:5) and MeCN−H_2O (6:13) and finally purified by HPLC using MeCN−H_2O (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; $[\alpha]_D^{23}$ +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 (C_5D_5N, 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 × 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 $C_{35}H_{55}O_8$, 603.3897).

Reduction of 1. $NaBH_4$ (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 H_2O (3 mL), washed successively with EtOAc (3 mL × 3), and concentrated under reduced pressure, it was purified by CC on silica gel using $CHCl_3$−MeOH (9:1) to give **12** (2.4 mg).

Compound 2: amorphous solid; $[\alpha]_D^{22}$ +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 (C_5D_5N, 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 $C_{35}H_{54}O_9Na$, 641.3666).

Reduction of 2. Compound **2** (5.1 mg) in MeOH (1 mL) was treated with $NaBH_4$ (10.0 mg), and a workup similar to that for **1** gave **17** (8.4 mg).

Compound 3: amorphous solid; $[\alpha]_D^{23}$ +34.0 (*c* 0.10, MeOH); IR (film) ν_{max} 3388 (OH), 2929 and 2878 (CH), 1774 (C=O) cm^{-1}; ^1H NMR (C_5D_5N, 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 $C_{41}H_{65}O_{14}$, 781.4374).

Table 2. ^{1}H 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	*J* (Hz)	position	^{1}H	*J* (Hz)	position	^{1}H	*J* (Hz)	position	^{1}H	*J* (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 H_2O followed by MeOH. The MeOH-eluted fraction was chromatographed on silica gel eluted with $CHCl_3$−MeOH (19:1) to yield **3a** (4.1 mg). The H_2O-eluted fraction (0.8 mg) was analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Japan); solvent, MeCN−H_2O (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; $[\alpha]_D^{23}$ +60.0 (*c* 0.10, MeOH); IR (film) ν_{max} 3376 (OH), 2949 (CH), 1765 (C=O) cm^{-1}; ^{1}H NMR (C_5D_5N, 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); ^{13}C NMR, see Table 1; HRESITOFMS *m/z* 657.3610 [M + Na]$^+$ (calcd. for $C_{35}H_{54}O_{10}Na$, 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; $[\alpha]_D^{23}$ +28.0 (*c* 0.10, $CHCl_3$); IR (film) ν_{max} 3397 (OH), 2947 and 2866 (CH), 1759 (C=O) cm^{-1}; ^{1}H NMR (C_5D_5N, 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); ^{13}C NMR, see Table 1; HRESITOFMS *m/z* 503.3359 [M + H]$^+$ (calcd for $C_{30}H_{47}O_6$, 503.3373).

Compound 5: amorphous solid; $[\alpha]_D^{23}$ −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}; ^{1}H NMR (C_5D_5N, 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); ^{13}C NMR, see Table 1; HRESITOFMS *m/z* 641.3694 [M + Na]$^+$ (calcd. for $C_{35}H_{54}O_9Na$, 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; $[\alpha]_D^{22}$ −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}; ^{1}H NMR (C_5D_5N, 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); ^{13}C NMR, see Table 1; HRESITOFMS *m/z* 487.3462 [M + H]$^+$ (calcd. for $C_{30}H_{47}O_5$, 487.3424).

Compound 6: amorphous solid; $[\alpha]_D^{26}$ −10.7 (*c* 0.31, MeOH); IR (film) ν_{max} 3404 (OH), 2939 (CH), 1642 (C=O) cm^{-1}; ^{1}H NMR (C_5D_5N, 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); ^{13}C NMR, see Table 1; HRESITOFMS *m/z* 791.4556 [M + Na]$^+$ (calcd for $C_{41}H_{68}O_{13}Na$, 791.4558).

Acid Hydrolysis of 6. A solution of **6** (5.8 mg) in 1 M HCl (dioxane−H_2O, 1:1; 2 mL) was heated at 95 °C for 2 h under an Ar

atmosphere. After cooling, the reaction mixture was diluted with H_2O (6 mL) and extracted with $CHCl_3$ (5 mL × 3). TLC analysis of the $CHCl_3$-soluble fraction showed that the labile aglycone decomposed under acidic conditions. The H_2O-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 (C_5D_5N, 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); ^{13}C NMR, see Table 1; HRESITOFMS *m/z* 751.4648 $[M + H]^+$ (calcd. for $C_{41}H_{67}O_{12}$, 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 (C_5D_5N, 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); ^{13}C NMR, see Table 1; HRESITOFMS *m/z* 783.4514 $[M + H]^+$ (calcd. for $C_{41}H_{67}O_{14}$, 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 (C_5D_5N, 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; ^{13}C NMR, see Table 1; HRESITOFMS *m/z*: 783.4579 $[M + H]^+$ (calcd for $C_{41}H_{67}O_{14}$, 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 (C_5D_5N, 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; ^{13}C NMR, see Table 1; HRESITOFMS *m/z* 967.4857 $[M + Na]^+$ (calcd for $C_{47}H_{76}O_{19}Na$, 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 × 10^4 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% CO_2/air for 24 h at 37 °C. After incubation, 4 μL of an EtOH−H_2O (1:1) solution containing the sample was added to give the final concentrations of 0.1−20 μg/mL, and 4 μL of EtOH−H_2O (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% CO_2/air for 4 h at 37 °C. The plate was then centrifuged at 1500*g* 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.

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Supporting Information Available: ^1H and ^{13}C 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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