# Triterpene Saponins from the Roots of *Clematis chinensis*

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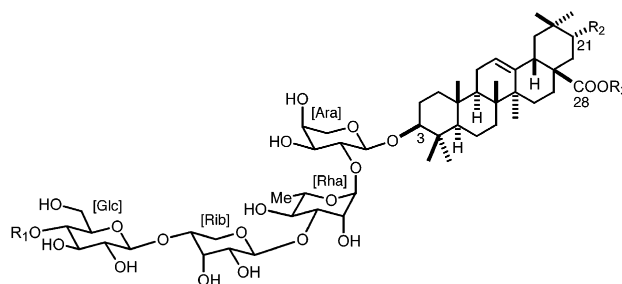
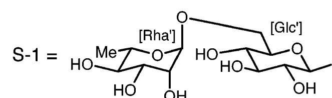
*Received April 1, 2004*

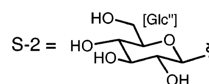
A saponin-enriched fraction prepared from the MeOH extract of the roots of *Clematis chinensis* showed cytotoxic activity against HL-60 promyelocytic leukemia cells, from which five new triterpene saponins (**1**−**5**) based on oleanolic acid, along with three known saponins (**6**−**8**), were isolated. The structures of the new saponins were determined on the basis of spectroscopic analysis, including extensive 1D and 2D NMR data and hydrolysis followed by chromatographic and spectroscopic analysis. Among the isolated saponins, monodesmosidic saponins exhibited cytotoxic activities against cultured tumor cells.

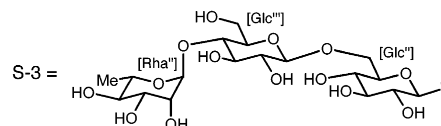
There are about 250 species belonging to the genus *Clematis* (Ranunculaceae) in the world. *Clematis chinensis* Osbeck is native to People's Republic of China, and its roots have long been used as an analgesic, a diuretic, an antitumor, and an anti-inflammatory agent in traditional Chinese medicine.[1] Some pharmacological studies of this crude drug showed that it had a significant hepatic protective and hypotensive effects.[2,3] The roots of *C. chinensis* are rich in saponins, and Kizu and Tomimori isolated more than 20 prosapogenins from the alkaline-treated crude saponin fraction of the roots extract.[4−8] Recently, three new bisdesmosidic triterpene saponins named clematichinenosides A−C have been isolated from the intact saponin fraction of *C. chinensis* roots.[9,10] During the course of a systematic survey of biologically active triterpene saponins from higher plants,[11−22] we have found that a saponin-enriched fraction prepared from the MeOH extract of *C. chinensis* roots showed cytotoxic activity against HL-60 human promyelocytic leukemia cells. Chromatographic separation of the saponin fraction has resulted in the isolation of five new triterpene saponins with up to a total of nine monosaccharides (**1**−**5**), along with three known triterpene saponins (**6**−**8**). This paper reports the structural determination of the new saponins (**1**−**5**) on the basis of spectroscopic analysis, including extensive 1D and 2D NMR data and hydrolysis followed by chromatographic and spectroscopic analysis, and also the cytotoxic activities of the isolated saponins.




| | R1 | R2 | R3 |
|---|---|---|---|
| **1** | S-1 | H | H |
| **2** | S-1 | H | S-2 |
| **3** | H | H | S-2 |
| **4** | H | OH | S-3 |
| **5** | S-1 | H | S-3 |

S-1 = 


S-2 = 


S-3 = 


## Results and Discussion

The dry roots of *C. chinensis* (5.0 kg) were extracted with hot MeOH. The MeOH extract was passed through a porous-polymer resin (Diaion HP-20) column and divided into 30% MeOH, 60% MeOH, 80% MeOH, MeOH, EtOH, and EtOAc eluate fractions. The MeOH eluate fraction exhibited a potent HL-60 cell growth inhibitory activity (98.6% inhibition at a sample concentration of 10 $\mu$g/mL; $IC_{50}$ 1.4 $\mu$g/mL) and was subjected to column chromatography over silica gel and octadecylsilanized (ODS) silica gel, giving **1**−**8**. Compounds **6**−**8** were identified as 3$\beta$-[(*O*-$\beta$-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**6**),[5] 3$\beta$-[(*O*-$\beta$-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl ester (**7**),[10] and 3$\beta$-[(*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl ester (**8**),[10] respectively.

Compound **1** was obtained as an amorphous solid, $[\alpha]^{25}_{D}$ −82.0° (MeOH). The high-resolution (HR) FABMS (positive mode) of **1** showed an accurate $[M + Na]^+$ ion peak at *m/z* 1359.6653 in accordance with an empirical molecular formula of $C_{64}H_{104}O_{29}$, which was supported by the $^{13}C$ NMR spectrum showing a total of 64 signals and various DEPT data (C × 8, CH × 33, $CH_2$ × 14, and Me × 9). The IR spectrum of **1** showed a broad absorption band for

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10.1021/np040088k CCC: $27.50 © 2004 American Chemical Society and American Society of Pharmacognosy
Published on Web 07/29/2004

**Table 1.** $^{1}$H and $^{13}$C NMR Data for the Glycosidic Moiety of **1** in Pyridine-$d_5$ at 318 K

| | position | $^{1}$H | | *J* (Hz) | $^{13}$C | | position | $^{1}$H | | *J* (Hz) | $^{13}$C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ara | 1 | 4.86 | d | 6.0 | 105.1 | Glc | 1 | 4.97 | d | 7.8 | 103.0 |
| | 2 | 4.57 | dd | 7.5, 6.0 | 75.7 | | 2 | 3.88 | dd | 9.0, 7.8 | 74.1 |
| | 3 | 4.28 | dd | 7.5, 4.0 | 74.4 | | 3 | 4.21 | dd | 9.0, 9.0 | 76.3 |
| | 4 | 4.27 | br s | | 69.2 | | 4 | 4.17 | dd | 9.0, 9.0 | 81.8 |
| | 5a | 4.32 | m | | 65.5 | | 5 | 3.89 | br dd | 9.0, 2.8 | 76.5 |
| | b | 3.84 | br d | 10.3 | | | 6a | 4.47 | dd | 11.4, 2.8 | 61.8 |
| | | | | | | | b | 4.43 | br d | 11.4 | |
| Rha | 1 | 6.24 | br s | | 101.5 | Glc′ | 1 | 5.12 | d | 7.8 | 104.9 |
| | 2 | 4.90 | br d | 2.9 | 71.9 | | 2 | 4.04 | dd | 9.0, 7.8 | 74.8 |
| | 3 | 4.69 | dd | 9.5, 2.9 | 82.0 | | 3 | 4.19 | dd | 9.0, 9.0 | 78.2 |
| | 4 | 4.44 | dd | 9.5, 9.5 | 72.7 | | 4 | 3.97 | dd | 9.0, 9.0 | 71.7 |
| | 5 | 4.63 | dq | 9.5, 6.1 | 69.8 | | 5 | 4.06 | ddd | 9.0, 5.6, 2.1 | 76.7 |
| | 6 | 1.56 | d | 6.1 | 18.4 | | 6a | 4.65 | dd | 10.5, 2.1 | 68.5 |
| | | | | | | | b | 3.96 | dd | 10.5, 5.6 | |
| Rib | 1 | 5.85 | d | 5.3 | 104.6 | Rha′ | 1 | 5.44 | d | 1.3 | 102.7 |
| | 2 | 4.12 | dd | 5.3, 3.2 | 72.5 | | 2 | 4.76 | dd | 3.4, 1.3 | 71.9 |
| | 3 | 4.67 | dd | 3.2, 3.2 | 69.2 | | 3 | 4.55 | dd | 9.1, 3.4 | 72.6 |
| | 4 | 4.34 | m | | 76.5 | | 4 | 4.24 | dd | 9.1, 9.1 | 74.1 |
| | 5a, b | 4.31 | m | | 61.6 | | 5 | 4.29 | dq | 9.1, 6.0 | 69.8 |
| | | | | | | | 6 | 1.60 | d | 6.0 | 18.6 |

hydroxyl groups at 3387 cm$^{-1}$, as well as absorption due to a carbonyl group at 1687 cm$^{-1}$. The $^{1}$H NMR spectrum of **1** measured in pyridine-$d_5$ showed signals for seven tertiary methyl groups at $\delta$ 1.31, 1.30, 1.13, 1.02, 0.98, 0.97, and 0.84 (each s) and a trisubstituted olefinic proton at $\delta$ 5.48 (t-like, $J$ = 3.2 Hz), which was typical of the oleanolic acid skeleton. In addition, signals for six anomeric protons were observed at $\delta$ 6.24 (br s), 5.85 (d, $J$ = 5.3 Hz), 5.44 (d, $J$ = 1.3 Hz), 5.12 (d, $J$ = 7.8 Hz), 4.97 (d, $J$ = 7.8 Hz), and 4.86 (d, $J$ = 6.0 Hz). The two three-proton doublet signals at $\delta$ 1.60 (d, $J$ = 6.0 Hz) and 1.56 (d, $J$ = 6.1 Hz) indicated the presence of two deoxyhexopyranosyl units in **1**. Acid hydrolysis of **1** with 1 M HCl in dioxane−$H_2O$ (1:1) gave olean-12-en-28-oic acid (oleanolic acid), together with L-arabinose, D-glucose, L-rhamnose, and D-ribose. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate, which was performed on an aminopropyl-bonded silica gel column using MeCN−$H_2O$ (3:1) as solvent system, with detection being carried out by using a combination of RI and optical rotation (OR) detectors. In the $^{13}$C NMR spectrum of **1**, the C-3 and C-28 carbon signals were observed at $\delta$ 88.7 and 180.2, respectively, implying that no sugar linkage was formed at the C-28 carboxyl group and that a hexaglycoside was attached to the C-3 hydroxyl group of the aglycone. The negative-ion FABMS showed an $[M - H]^-$ at $m/z$ 1335 and prominent fragments at $m/z$ 1189 $[(M - H) - 146]^-$ (cleavage of one deoxyhexose unit), 1027 $[(M - H) - 146 - 162]^-$ (cleavage of one deoxyhexose unit and one hexose unit), 865 $[(M - H) - 146 - 162 \times 2]^-$ (cleavage of one deoxyhexose unit and two hexose units), 733 $[(M - H) - 146 - 162 \times 2 - 132]^-$ (cleavage of one deoxyhexose unit, two hexose units, and one pentose unit), and 587 $[(M - H) - 146 \times 2 - 162 \times 2 - 132]^-$ (cleavage of two deoxyhexose units, two hexose units, and one pentose unit). A peak at $m/z$ 455 was assigned to the aglycone moiety. This simple fragment ion pattern and the results of acid hydrolysis suggested that **1** had a linear sugar sequence of rhamnosyl-glucosyl-glucosyl-ribosyl-rhamnosyl-arabinosyl or rhamnosyl-glucosyl-glucosyl-arabinosyl-rhamnosyl-ribosyl in order of the terminal unit to the inner unit attached to the aglycone. The exact sugar sequence and its linkage position to the aglycone were solved by detailed analysis of the 1D TOCSY and 2D NMR spectra. The $^{1}$H NMR subspectra of individual monosaccharide units were obtained by using selective irradiation of the easily identifiable anomeric proton signals, as well as irradiation of other nonoverlapping proton signals in a series of 1D TOCSY experiments. Subsequent analysis of the $^{1}$H−$^{1}$H COSY spectrum resulted in the sequential assignments of all the proton resonances due to the six monosaccharides, including identification of most of their multiplet patterns and coupling constants as shown in Table 1. The HSQC spectrum correlated the proton resonances with those of the corresponding one-bond coupled carbons and the HSQC-TOCSY spectrum associated the anomeric protons with their respective skeleton carbon atoms, leading to unambiguous assignments of the carbon shifts (Table 1). Comparison of the carbon chemical shifts thus assigned with those of the reference methyl glycosides,[23,24] taking into account the known effects of *O*-glycosylation, indicated that **1** contained an α-L-rhamnopyranosyl unit (Rha′) as the terminal glycosyl moiety and an α-L-arabinopyranosyl unit (Ara), two $\beta$-D-glucopyranosyl units (Glc and Glc′), a $\beta$-D-ribopyranosyl unit (Rib), and an α-L-rhamnopyranosyl unit (Rha) as the substituted sugar moieties. The relatively large $^{3}J_{H-1,H-2}$ values of the arabinosyl, glucosyl, and ribosyl moieties (5.3−7.8 Hz) indicated an α anomeric orientation for the arabinosyl and $\beta$ for the glucosyls and ribosyl. The large $^{1}J_{H-1,C-1}$ values of the rhamnosyl moieties (Rha, 174 Hz; Rha′, 168 Hz) confirmed that the anomeric protons were equatorial (α-pyranoid anomeric form).[25] Finally, the $^{3}J_{C,H}$ correlations from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide and the aglycone revealed the sugar sequence. In the HMBC spectrum, the anomeric proton signals at $\delta$ 6.24 (Rha), 5.85 (Rib), 5.44 (Rha′), 5.12 (Glc′), 4.97 (Glc), and 4.86 (Ara) showed long-range correlations with C-2 of Ara at $\delta$ 75.7, C-3 of Rha at $\delta$ 82.0, C-6 of Glc′ at $\delta$ 68.5, C-4 of Glc at $\delta$ 81.8, C-4 of Rib at $\delta$ 76.5, and C-3 of the aglycone at $\delta$ 88.7, respectively. All of these data were consistent with the structure 3$\beta$-[(*O*-α-L-rhamnopyranosyl-(1→6)-*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid, which was assigned to **1**.

Compound **2** was isolated as an amorphous solid with a molecular formula of $C_{70}H_{114}O_{34}$, as determined from data of the positive-ion FABMS ($m/z$ 1521 $[M + Na]^+$), negative-ion FABMS ($m/z$ 1497 $[M - H]^-$), $^{13}$C NMR spectrum (70 carbon signals), and elemental analysis. The $^{1}$H NMR spectrum of **2** showed signals for seven anomeric protons at $\delta$ 6.31 (d, $J$ = 8.1 Hz), 6.24 (br s), 5.85 (d, $J$ = 5.4 Hz),

5.42 (d, $J = 1.4$ Hz), 5.10 (d, $J = 7.8$ Hz), 4.95 (d, $J = 7.8$ Hz), and 4.84 (d, $J = 6.0$ Hz), along with signals for seven tertiary methyl groups at $\delta$ 1.28, 1.25, 1.13, 1.07, 0.91, 0.88, and 0.85 (each s) and a trisubstituted olefinic proton at $\delta$ 5.42 (t-like, $J = 3.1$ Hz), as observed for **1**. Acid hydrolysis of **2** yielded oleanolic acid, L-arabinose, D-glucose, L-rhamnose, and D-ribose. Comparison of the $^{1}$H and $^{13}$C NMR spectra of **2** with those of **1** revealed that **2** had a terminal $\beta$-D-glucopyranosyl moiety (Glc″) in addition to a hexaglycoside group attached to C-3 of the aglycone, which was identical to that of **1**. Since the C-28 carbonyl carbon signal of the aglycone of **2** was shifted upfield by 3.8 ppm in comparison with that of **1**, the additional glucosyl moiety was presumed to be located at C-28 in an ester linkage form. This was confirmed by alkaline hydrolysis of **2** with 6% KOH (EtOH−$H_2O$, 1:1), giving **1**, and by an HMBC correlation from the anomeric proton of Glc″ at $\delta$ 6.31 to C-28 of the aglycone at $\delta$ 176.4. Accordingly, the structure of **2** was determined as 3$\beta$-[(*O*-α-L-rhamnopyranosyl-(1→6)-*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid $\beta$-D-glucopyranosyl ester.

Compound **3** was analyzed for $C_{58}H_{94}O_{25}$ by combined positive-ion FABMS (*m/z* 1213 [M + Na]$^+$), $^{13}$C NMR with DEPT data, and elemental analysis. The $^{1}$H NMR spectrum of **3** showed five anomeric proton signals at $\delta$ 6.28 (d, $J =$ 8.1 Hz), 6.19 (br s), 5.83 (d, $J = 5.5$ Hz), 4.98 (1H, d, $J =$ 7.8 Hz), and 4.81 (d, $J = 6.0$ Hz), along with seven tertiary methyl and one olefinic proton signals characteristic of oleanolic acid. The $^{13}$C NMR spectroscopic features were also suggestive of oleanolic acid 3,28-bisdesmoside. Acid hydrolysis of **3** with 1 M HCl furnished oleanolic acid, L-arabinose, D-glucose, L-rhamnose, and D-ribose, whereas alkaline treatment of **3** with 6% KOH (EtOH−$H_2O$, 1:1) yielded a known oleanolic acid tetraglycoside (**9**),[4,10] which was identical to the compound produced by alkaline treatment of **8**. These facts indicated that the tetraglycoside linked to C-3 of the aglycone of **3** was the same as that of **8**. When the $^{13}$C NMR spectrum of **3** was compared with that of **9**, a set of additional six signals corresponding to a terminal $\beta$-D-glucosyl group appeared, and an HMBC correlation was observed between the signals of the anomeric proton of the glucosyl group ($\delta$ 6.28) and the C-28 carbonyl carbon of the aglycone ($\delta$ 176.4). Accordingly, the structure of **3** was assigned as 3$\beta$-[(*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-$\beta$-D-glucopyranosyl ester.

Compound **4** had the molecular formula $C_{70}H_{114}O_{35}$ on the basis of the positive-ion FABMS (*m/z* 1537 [M + Na]$^+$), negative-ion FABMS (*m/z* 1513 [M − H]$^-$), $^{13}$C NMR spectrum, and elemental analysis. The $^{1}$H NMR spectrum contained seven three-proton singlet signals between $\delta$ 1.34 and 0.87, an olefinic proton signal at $\delta$ 5.48 (t-like, $J = 3.1$ Hz), and seven anomeric proton signals. Comparison of the $^{1}$H and $^{13}$C NMR spectra of **4** with those of **8** showed their considerable structural similarity. Acid hydrolysis of **4** with 1 M HCl resulted in the production of an aglycone (**4a**), together with L-arabinose, D-glucose, L-rhamnose, and D-ribose. The spectroscopic properties of **4a** were closely related to those of oleanolic acid; however, the molecular formula of **4a** ($C_{30}H_{48}O_4$) was higher by one oxygen atom than that of oleanolic acid, implying the presence of one more hydroxyl group in addition to the C-3$\beta$ hydroxyl group. The hydroxymethine proton at $\delta$ 3.81 (dd, $J = 2.7$, 2.7 Hz) showed spin-couplings with the methylene protons at $\delta$ 2.39 (dd, $J = 14.4$, 2.7 Hz, H-22a) and 2.34 (dd, $J =$ 14.4, 2.7 Hz, H-22b) in the $^{1}$H−$^{1}$H COSY spectrum and HMBC correlations with C-17 ($\delta$ 47.2), C-19 ($\delta$ 41.7), C-20 ($\delta$ 35.9), C-29 ($\delta$ 28.5), and C-30 ($\delta$ 25.1), indicating the presence of a hydroxyl group at C-21. NOE correlations from H-21 to both Me-29 and Me-30 confirmed the C-21α configuration. The downfield shift of the H-19 axial proton [$\delta$ 2.62 (dd, $J = 13.9$, 13.9 Hz)] and upfield shift of the C-19 carbon ($\delta$ 41.7) were consistent with the presence of the C-21α-axial hydroxyl group. The structure of **4a** was determined to be 21α-hydroxyoleanolic acid. Although **4a** has been reported from *Olax dissitiflora*,[26] *Amaracus dictamnus*,[27] *Origanum compactum*,[28] and *Mentha citrata*,[29] no spectroscopic data for intact **4a** are available. The IR, $^{1}$H NMR, and $^{13}$C NMR data for **4a** are reported in Table 2 and the Experimental Section. As for the sugar sequences of **4**, the tetraglycoside and the triglycoside, which were attached to C-3 and C-28 of the aglycone, respectively, were suggested to be identical with those of **8**. This was confirmed by analysis of the HMBC spectrum of **4**, resulting in the detection of the $^{3}J_{C,H}$ correlations from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide or the aglycone. The structure of **4** was thus defined as 3$\beta$-[(*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-21α-hydroxyolean-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl ester.

Compound **5** was the most polar triterpene saponin, and its molecular formula was derived as $C_{82}H_{134}O_{43}$ from the positive-ion FABMS (*m/z* 1829 [M + Na]$^+$), negative-ion FABMS (*m/z* 1805 [M − H]$^-$), $^{13}$C NMR, and elemental analysis. The $^{1}$H NMR spectrum of **5** displayed signals for nine anomeric protons at $\delta$ 6.22 (br s), 6.20 (d, $J = 8.1$ Hz), 5.83 (br s), 5.81 (d, $J = 5.5$ Hz), 5.40 (br s), 5.11 (d, $J = 7.8$ Hz), 4.99 (d, $J = 7.8$ Hz), 4.95 (d, $J = 7.8$ Hz), and 4.83 (d, $J = 5.9$ Hz), as well as signals for seven tertiary methyl

**Table 2.** $^{13}$C NMR Data for the Aglycone Moiety of **1**−**4**, **4a**, and **5** in Pyridine-$d_5$

| position | **1** | **2** | **3** | **4** | **4a** | **5** |
|---|---|---|---|---|---|---|
| 1 | 38.8 | 38.9 | 38.8 | 38.9 | 39.0 | 38.8 |
| 2 | 26.6 | 26.6 | 26.5 | 26.6 | 28.1 | 26.6 |
| 3 | 88.7 | 88.7 | 88.6 | 88.7 | 78.1 | 88.6 |
| 4 | 39.5 | 39.6 | 39.5 | 39.5 | 39.4 | 39.5 |
| 5 | 56.0 | 56.0 | 55.9 | 56.0 | 55.9 | 55.9 |
| 6 | 18.5 | 18.5 | 18.5 | 18.4 | 18.8 | 18.4 |
| 7 | 33.1 | 33.1 | 33.0 | 33.1 | 33.5 | 32.9 |
| 8 | 39.7 | 39.9 | 39.8 | 39.7 | 39.7 | 39.8 |
| 9 | 48.0 | 48.0 | 47.9 | 48.1 | 48.2 | 47.9 |
| 10 | 37.0 | 37.0 | 36.9 | 37.0 | 37.4 | 36.9 |
| 11 | 23.7 | 23.8 | 23.7 | 23.8 | 23.9 | 23.7 |
| 12 | 122.5 | 122.8 | 122.7 | 122.7 | 122.5 | 122.7 |
| 13 | 144.8 | 144.1 | 144.1 | 144.3 | 145.1 | 144.1 |
| 14 | 42.1 | 42.1 | 42.0 | 42.4 | 42.6 | 42.0 |
| 15 | 28.3 | 28.2 | 28.1 | 28.7 | 28.8 | 28.1 |
| 16 | 23.6 | 23.4 | 23.3 | 26.9 | 27.4 | 23.2 |
| 17 | 46.6 | 47.0 | 46.9 | 47.4 | 47.2 | 46.9 |
| 18 | 41.9 | 41.7 | 41.6 | 41.7 | 42.1 | 41.5 |
| 19 | 46.4 | 46.2 | 46.1 | 41.3 | 41.7 | 46.1 |
| 20 | 30.9 | 30.7 | 30.6 | 35.6 | 35.9 | 30.6 |
| 21 | 34.2 | 34.0 | 33.9 | 73.4 | 73.8 | 33.9 |
| 22 | 33.2 | 32.5 | 32.4 | 39.6 | 40.4 | 32.4 |
| 23 | 28.1 | 28.1 | 28.0 | 28.1 | 28.8 | 28.0 |
| 24 | 17.1 | 17.1 | 17.0 | 17.1 | 16.6 | 17.0 |
| 25 | 15.5 | 15.6 | 15.5 | 15.7 | 15.7 | 15.6 |
| 26 | 17.3 | 17.4 | 17.3 | 17.5 | 17.6 | 17.4 |
| 27 | 26.1 | 26.1 | 26.0 | 25.6 | 25.7 | 26.0 |
| 28 | 180.2 | 176.4 | 176.4 | 176.5 | 180.3 | 176.5 |
| 29 | 33.2 | 33.1 | 33.0 | 28.3 | 28.5 | 33.0 |
| 30 | 23.7 | 23.6 | 23.5 | 24.9 | 25.1 | 23.6 |

groups and an olefinic proton due to the oleanolic acid moiety. The C-3 and C-28 bisdesmosidic nature of **5** was shown by the $^{13}$C NMR shifts of C-3 ($\delta$ 88.6) and C-28 ($\delta$ 176.5). Acid hydrolysis of **5** with 1 M HCl furnished oleanolic acid, L-arabinose, D-glucose, L-rhamnose, and D-ribose, whereas alkaline hydrolysis with 6% KOH (EtOH−$H_2O$, 1:1) yielded **1**. The triglycoside attached to C-28 was presumed to be the same as that of **4** and **8**, confirmative evidence for which was obtained by HMBC correlations from the anomeric protons assigned for the triglycoside moiety to their respective linkage carbons. The structure of **5** was elucidated as 3$\beta$-[(*O*-$\alpha$-L-rhamnopyranosyl-(1→6)-*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-ribopyranosyl-(1→3)-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-$\alpha$-L-rhamnopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl ester.

Compounds **1**−**8** were evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells using an MTT assay method.[30] The monodesmosidic saponins (**1** and **6**) showed cytotoxic activity against HL-60 cells with the $IC_{50}$ values of 2.8 and 2.3 $\mu$M, respectively. Although the main saponin constituent **5** of *C. chinensis* roots did not exhibit any apparent cytotoxicity even at a concentration of 20 $\mu$M, it can be converted to the cytotoxic saponin **1** by cleavage of the C-28 triglycosyl ester linkage and is concluded to have a cytotoxic potentiality. Compound **1** exhibited no significant differential cellular sensitivities when **1** was evaluated in the Japanese Foundation for Cancer Research 39 cell line assay.[31] However, BSY-1 breast cancer cells, U251 and SF-295 CNS cancer cells, and PC-3 prostate cancer cells were relatively sensitive to **1** with respective $LC_{50}$ values of 5.9, 6.3, 6.0, and 6.0 $\mu$M, and NCI-H460 lung cells, OVCAR-3 and OVCAR-8 ovarian cells, and stomach MKN28 cells were relatively resistant, with respective $LC_{50}$ values of 40, 47, 71, and 30 $\mu$M.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured by using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for $^1$H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as $\delta$ values with reference to tetramethylsilane (TMS) as internal standard. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA) mass spectrometer, using a dithiothreitol and dithioerythritol (3:1) matrix. Elemental analyses were carried out using an Elemental Vario EL (Hanau, Germany) elemental analyzer. Silica gel (Fuji-Silysia Chemical, Aichi, Japan), ODS silica gel (Nacalai Tesque, Kyoto, Japan), and Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F254 (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F254S (0.25 mm, Merck) plates, and spots were visualized by spraying the plates with 10% $H_2SO_4$ solution, followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh), a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port with a 20 $\mu$L sample loop. A Kaseisorb NH2-60-5 LC column (4.6 mm i.d. × 250 mm, 5 $\mu$m, Tokyo-Kasei, Tokyo, Japan) was employed for HPLC analysis. The following reagents were obtained from the indicated companies: RPMI 1640 medium (Gibco, Gland Island, NY); FBS (Bio-Whittaker, Walkersville, MD); MTT (Sigma, St. Louis, MO); penicillin and streptomycin (Meiji-Seika, Tokyo, Japan). All other chemicals used were of biochemical reagent grade.

**Plant Material.** The plant material defined as the roots of *C. chinensis* was obtained from a wholesale firm in Uchida-Wakanyaku, Tokyo, Japan, and authenticated by one of the authors (Y.S.). A small amount of the sample is preserved in our laboratory (00-CC-UW-07).

**Extraction and Isolation.** The plant material (5.0 kg) was extracted with hot MeOH (3L × 3). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (432 g) was passed through a Diaion HP-20 column, successively eluting with 30% MeOH, 60% MeOH, 80% MeOH, MeOH, EtOH, and EtOAc. The MeOH eluate fraction (39 g) exhibited a potent cytotoxic activity against HL-60 cells (98.6% cell growth inhibition at a sample concentration of 10 $\mu$g/mL; $IC_{50}$ 1.4 $\mu$g/mL). Column chromatography of the MeOH eluate portion on silica gel and elution with a stepwise gradient mixture of $CHCl_3$−MeOH (4:1; 2:1; 1:1) and finally with MeOH gave five fractions I−V. Fraction I was subjected to a silica gel column eluting with $CHCl_3$−MeOH−$H_2O$ (40:10:1) to give **6** (19.8 mg). Fraction II was purified by silica gel column chromatography eluting with $CHCl_3$−MeOH−$H_2O$ (7:4:1) and ODS silica gel column chromatography with MeOH−$H_2O$ (4:1) to yield **3** (14.2 mg). Fraction III was separated by column chromatography on silica gel eluting with $CHCl_3$−MeOH−$H_2O$ (7:4:1) and ODS silica gel with MeOH−$H_2O$ (8:5) to afford **1** (435 mg). Fraction IV was subjected to a silica gel column eluting with $CHCl_3$−MeOH−$H_2O$ (7:4:1) and an ODS silica gel column with MeOH−$H_2O$ (8:5) and MeCN−$H_2O$ (5:8) to obtain **2** (21.1 mg), **4** (119 mg), **7** (32.1 mg), and **8** (627 mg). Fraction V was chromatographed on silica gel eluting with $CHCl_3$−MeOH−$H_2O$ (7:4:1) and ODS silica gel with MeOH−$H_2O$ (8:5) to furnish **5** (5.41 g).

**Compound 1:** amorphous solid; $[\alpha]_D^{25}$ −82.0° (*c* 0.25, MeOH); IR (film) $\nu_{max}$ 3387 (OH), 2941 (C−H), 1687 (C=O), 1057 cm$^{-1}$; $^1$H NMR (pyridine-$d_5$) $\delta$ 5.48 (1H, t-like, $J$ = 3.2 Hz), 3.29 (1H, overlapping, H-18), 3.28 (1H, dd, $J$ = 11.1, 4.1 Hz, H-3), 1.31 (3H, s, Me-27), 1.30 (3H, s, Me-23), 1.13 (3H, s, Me-24), 1.02 (3H, s, Me-30), 0.98 (3H, s, Me-26), 0.97 (3H, s, Me-29), 0.84 (3H, s, Me-25), signals for the sugar moiety, see Table 2; $^{13}$C NMR, see Tables 1 and 2; HRFABMS (positive mode) *m/z* 1359.6653 [M + Na]$^+$ (calcd for $C_{64}H_{104}O_{29}Na$, 1359.6561); FABMS (negative mode) *m/z* 1335 [M − H]$^-$, 1189 [(M − H) − deoxyhexosyl]$^-$, 1027 [(M − H) − deoxyhexosyl − hexosyl]$^-$, 865 [(M − H) − deoxyhexosyl − hexosyl × 2]$^-$, 733 [(M − H) − deoxyhexosyl − hexosyl × 2 − pentosyl]$^-$, 587 [(M − H) − deoxyhexosyl × 2 − hexosyl × 2 − pentosyl]$^-$, 455.

**Acid Hydrolysis of 1.** A solution of **1** (45 mg) in 1 M HCl (dioxane−$H_2O$, 1:1, 4 mL) was heated at 90 °C for 1 h under an Ar atmosphere. After cooling, the mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on silica gel eluting with $CHCl_3$−MeOH (99:1; 1:1) to give oleanolic acid (11.4 mg) and a sugar fraction (7.1 mg). The sugar fraction was passed through a Sep-Pak C18 cartridge (Waters, Milford, MA) and a Toyopak IC-SP M cartridge (Tosoh), which was then analyzed by HPLC under the following conditions: column, Kaseisorb NH2-60-5 LC (4.6 mm i.d. × 250 mm, 5 $\mu$m); solvent, MeCN−$H_2O$ (3:1); flow rate, 0.6 mL/min; detection, RI and OR. The identification of L-arabinose, D-glucose, L-rhamnose, and D-ribose present in the sugar fraction was carried out by comparison of their retention times and polarities with those of authentic samples; $t_R$ (min) 10.49 (L-rhamnose, negative polarity), 10.90 (D-ribose, negative polarity), 13.26 (L-arabinose, positive polarity), 16.04 (D-glucose, positive polarity).

**Compound 2:** amorphous solid; $[\alpha]_D^{25}$ −70.0° (*c* 0.25, MeOH); IR (film) $\nu_{max}$ 3387 (OH), 2940 (C−H), 1746 (C=O), 1067 cm$^{-1}$; $^1$H NMR (pyridine-$d_5$) $\delta$ 6.31 (1H, d, $J$ = 8.1 Hz, H-1 of Glc″), 6.24 (1H, br s, H-1 of Rha), 5.85 (1H, d, $J$ = 5.4 Hz, H-1 of Rib), 5.42 (1H, d, $J$ = 1.4 Hz, H-1 of Rha′), 5.42 (1H, t-like, $J$ = 3.1 Hz, H-12), 5.10 (1H, d, $J$ = 7.8 Hz, H-1 of Glc′), 4.95 (1H, d, $J$ = 7.8 Hz, H-1 of Glc), 4.84 (1H, d, $J$ = 6.0 Hz, H-1 of Ara), 3.27 (1H, dd, $J$ = 11.7, 4.1 Hz, H-3), 3.18 (1H, dd, $J$ = 13.6, 3.7 Hz, H-18), 1.58 (3H, d, $J$ = 5.8 Hz, Me-6 of Rha′), 1.53 (3H, d, $J$ = 6.1 Hz, Me-6 of Rha), 1.28 (3H, s, Me-23), 1.25 (3H, s, Me-27), 1.13 (3H, s, Me-24), 1.07 (3H, s, Me-26), 0.91 (3H, s, Me-29), 0.88 (3H, s, Me-30), 0.85 (3H, s, Me-

**Table 3.** $^{13}C$ NMR Data for the Glycosidic Moieties of **2**−**5** in Pyridine-$d_5$

| | position | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| Ara | 1 | 105.2 | 105.1 | 105.2 | 105.1 |
| | 2 | 75.4 | 75.4 | 75.3 | 75.3 |
| | 3 | 74.6 | 74.4 | 74.6 | 74.5 |
| | 4 | 69.3 | 69.2 | 69.3 | 69.2 |
| | 5 | 65.6 | 65.5 | 65.6 | 65.5 |
| Rha | 1 | 101.4 | 101.3 | 101.3 | 101.3 |
| | 2 | 71.9 | 71.8 | 71.9 | 71.8 |
| | 3 | 82.0 | 81.8 | 81.9 | 81.8 |
| | 4 | 72.7 | 72.6 | 72.7 | 72.6 |
| | 5 | 69.8 | 69.7 | 69.7 | 69.7 |
| | 6 | 18.4 | 18.4 | 18.4 | 18.4 |
| Rib | 1 | 104.7 | 104.4 | 104.6 | 104.4 |
| | 2 | 72.5 | 72.3 | 72.4 | 72.3 |
| | 3 | 69.4 | 69.4 | 69.5 | 69.4 |
| | 4 | 76.6 | 76.2 | 76.3 | 76.3 |
| | 5 | 61.6 | 61.7 | 61.7 | 61.6 |
| Glc | 1 | 103.1 | 103.3 | 103.4 | 103.0 |
| | 2 | 74.0 | 74.6 | 74.7 | 74.0 |
| | 3 | 76.3 | 78.1 | 78.2 | 76.2 |
| | 4 | 81.9 | 71.3 | 71.4 | 81.7 |
| | 5 | 76.5 | 78.5 | 78.5 | 76.4 |
| | 6 | 61.8 | 62.3 | 62.4 | 61.6 |
| Glc′ | 1 | 104.9 | | | 104.8 |
| | 2 | 74.8 | | | 74.8 |
| | 3 | 78.2 | | | 78.1 |
| | 4 | 71.7 | | | 71.7 |
| | 5 | 76.7 | | | 76.6 |
| | 6 | 68.6 | | | 68.5 |
| Rha′ | 1 | 102.7 | | | 102.6 |
| | 2 | 71.9 | | | 71.7 |
| | 3 | 72.6 | | | 72.6 |
| | 4 | 74.1 | | | 73.9 |
| | 5 | 69.8 | | | 69.7 |
| | 6 | 18.6 | | | 18.5 |
| Glc″ | 1 | 95.7 | 95.7 | 95.7 | 95.5 |
| | 2 | 74.1 | 74.0 | 73.8 | 73.7 |
| | 3 | 78.9 | 78.7 | 78.6 | 78.5 |
| | 4 | 71.1 | 71.0 | 70.7 | 70.6 |
| | 5 | 79.3 | 79.2 | 78.0 | 77.9 |
| | 6 | 62.2 | 62.1 | 69.2 | 69.0 |
| Glc‴ | 1 | | | 104.8 | 104.6 |
| | 2 | | | 75.2 | 75.2 |
| | 3 | | | 76.4 | 76.4 |
| | 4 | | | 78.1 | 78.1 |
| | 5 | | | 77.0 | 77.0 |
| | 6 | | | 61.2 | 61.1 |
| Rha″ | 1 | | | 102.6 | 102.6 |
| | 2 | | | 72.5 | 72.4 |
| | 3 | | | 72.7 | 72.5 |
| | 4 | | | 73.9 | 73.9 |
| | 5 | | | 70.2 | 70.2 |
| | 6 | | | 18.4 | 18.4 |

25); $^{13}C$ NMR, see Tables 1 and 3; FABMS (positive mode) *m/z* 1521 [M + Na]$^+$; FABMS (negative mode) *m/z* 1497 [M − H]$^-$, 1335 [(M − H) − hexosyl]$^-$, 1189 [(M − H) − hexosyl − deoxyhexosyl]$^-$, 1027 [(M − H) − hexosyl × 2 − deoxyhexosyl]$^-$, 865 [(M − H) − hexosyl × 3 − deoxyhexosyl]$^-$, 733 [(M − H) − hexosyl × 3 − deoxyhexosyl − pentosyl]$^-$; *anal.* C 53.05%, H 7.81%, calcd for $C_{70}H_{114}O_{34}\cdot 5H_2O$, C 52.84%, H 7.86%.

**Acid Hydrolysis of 2.** Compound **2** (6 mg) was subjected to acid hydrolysis as described for **1** to give oleanolic acid (3.1 mg) and a sugar fraction (2.4 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-arabinose, D-glucose, L-rhamnose, and D-ribose.

**Alkaline Hydrolysis of 2.** Compound **2** (5 mg) was treated with 6% KOH in EtOH−$H_2O$ (1:1, 4 mL) at 95 °C for 1 h under an Ar atmosphere. The mixture was neutralized by passage through an Amberlite IR-120B column (Organo, Tokyo, Japan) and then chromatographed over silica gel eluting with $CHCl_3$−MeOH−$H_2O$ (7:4:1) to yield **1** (2.8 mg).

**Compound 3:** amorphous solid; $[\alpha]_D^{25}$ −38.0° (*c* 0.10, MeOH); IR (film) $\nu_{max}$ 3376 (OH), 2941 (C−H), 1745 (C=O), 1070 cm$^{-1}$; $^1H$ NMR (pyridine-$d_5$) $\delta$ 6.28 (1H, d, $J$ = 8.1 Hz, H-1 of Glc″), 6.19 (1H, br s, H-1 of Rha), 5.83 (1H, d, $J$ = 5.5 Hz, H-1 of Rib), 5.40 (1H, t-like, $J$ = 3.1 Hz, H-12), 4.98 (1H, d, $J$ = 7.8 Hz, H-1 of Glc), 4.81 (1H, d, $J$ = 6.0 Hz, H-1 of Ara), 3.24 (1H, dd, $J$ = 11.7, 4.1 Hz, H-3), 3.16 (1H, dd, $J$ = 13.6, 3.8 Hz, H-18), 1.51 (3H, d, $J$ = 6.2 Hz, Me-6 of Rha), 1.24 (3H × 2, s, Me-23 and Me-27), 1.09 (3H, s, Me-24), 1.05 (3H, s, Me-26), 0.89 (3H, s, Me-29), 0.86 (3H, s, Me-30), 0.82 (3H, s, Me-25); $^{13}C$ NMR, see Tables 1 and 3; FABMS (positive mode) *m/z* 1213 [M + Na]$^+$, 1051 [(M + Na) − hexosyl]$^+$; *anal.* C 56.80%, H 8.32%, calcd for $C_{58}H_{94}O_{25}\cdot 2H_2O$, C 56.76%, H 8.05%.

**Acid Hydrolysis of 3.** Compound **3** (5 mg) was subjected to acid hydrolysis as described for **1** to give oleanolic acid (2.3 mg) and a sugar fraction (1.7 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-arabinose, D-glucose, L-rhamnose, and D-ribose.

**Alkaline Hydrolysis of 3 and 8.** Compound **3** (5 mg) was treated with 6% KOH in EtOH−$H_2O$ (1:1, 3 mL) at 95 °C for 1 h under an Ar atmosphere. The mixture was neutralized by passage through an Amberlite IR-120B column and then chromatographed over silica gel eluting with $CHCl_3$−MeOH−$H_2O$ (40:10:1) to yield **9** (2.7 mg). Compound **8** (80 mg) was subjected to alkaline hydrolysis as described above to give **9** (42.7 mg).

**Compound 4:** amorphous solid; $[\alpha]_D^{25}$ −108.0° (*c* 0.25, MeOH); IR (film) $\nu_{max}$ 3387 (OH), 2937 (C−H), 1733 (C=O), 1065 cm$^{-1}$; $^1H$ NMR (pyridine-$d_5$) $\delta$ 6.25 (1H, d, $J$ = 8.1 Hz, H-1 of Glc″), 6.23 (1H, br s, H-1 of Rha), 5.82 (1H, d, $J$ = 1.5 Hz, Rha″), 5.81 (1H, d, $J$ = 5.8 Hz, H-1 of Rib), 5.48 (1H, t-like, $J$ = 3.1 Hz, H-12), 5.00 (1H, d, $J$ = 7.8 Hz, H-1 of Glc), 4.97 (1H, d, $J$ = 7.9 Hz, H-1 of Glc‴), 4.83 (1H, d, $J$ = 6.0 Hz, H-1 of Ara), 3.68 (1H, br s, H-21), 3.36 (1H, dd, $J$ = 14.1, 2.6 Hz, H-18), 3.27 (1H, dd, $J$ = 11.4, 3.8 Hz, H-3), 1.68 (3H, d, $J$ = 6.2 Hz, Me-6 of Rha″), 1.52 (3H, d, $J$ = 6.1 Hz, Me-6 of Rha), 1.34 (3H, s, Me-27), 1.26 (3H, s, Me-23), 1.18 (3H, s, Me-29), 1.12 (3H, s, Me-24), 1.08 (3H, s, Me-26), 1.00 (3H, s, Me-30), 0.87 (3H, s, Me-25); $^{13}C$ NMR, see Tables 1 and 3; FABMS (positive mode) *m/z* 1537 [M + Na]$^+$; FABMS (negative mode) *m/z* 1513 [M − H]$^-$; *anal.* C 53.65%, H 7.72%, calcd for $C_{70}H_{114}O_{35}\cdot 3H_2O$, C 53.52%, H 7.71%.

**Acid Hydrolysis of 4.** Compound **4** (57.2 mg) was subjected to acid hydrolysis as described for **1** to give **4a** (15.6 mg) and a sugar fraction (25.9 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-arabinose, D-glucose, L-rhamnose, and D-ribose.

**Compound 4a:** amorphous solid; $[\alpha]_D^{25}$ −46.4° (*c* 0.25, MeOH); IR (film) $\nu_{max}$ 3434 (OH), 2928 and 2871 (C−H), 1685 (C=O) cm$^{-1}$; $^1H$ NMR (pyridine-$d_5$) $\delta$ 5.60 (1H, t-like, $J$ = 3.2 Hz, H-12), 3.81 (1H, dd, $J$ = 2.7, 2.7 Hz, H-21), 3.53 (1H, dd, $J$ = 13.9, 3.2 Hz, H-18), 3.47 (1H, dd, $J$ = 10.6, 5.4 Hz, H-3), 2.62 (1H, dd, $J$ = 13.9, 13.9 Hz, H-19ax), 2.39 (1H, dd, $J$ = 14.4, 2.7 Hz, H-22a), 2.34 (1H, dd, $J$ = 14.4, 2.7 Hz, H-22b), 1.40 (3H, s, Me-27), 1.30 (1H, dd, $J$ = 13.9, 3.2 Hz, H-19eq), 1.27 (3H, s, Me-29), 1.25 (3H, s, Me-23), 1.16 (3H, s, Me-30), 1.08 (3H, s, Me-26), 1.04 (3H, s, Me-24), 0.94 (3H, s, Me-25); $^{13}C$ NMR, see Table 1; FABMS (positive mode) *m/z* 473 [M + H]$^+$; *anal.* C 74.64%, H 10.10%, calcd for $C_{30}H_{48}O_4\cdot 1/2H_2O$, C 74.80%, H 10.25%.

**Compound 5:** amorphous solid; $[\alpha]_D^{25}$ −94.0° (*c* 0.25, MeOH); IR (film) $\nu_{max}$ 3387 (OH), 2939 (C−H), 1733 (C=O), 1063 cm$^{-1}$; $^1H$ NMR (pyridine-$d_5$) $\delta$ 6.22 (1H, br s, H-1 of Rha), 6.20 (1H, d, $J$ = 8.1 Hz, H-1 of Glc″), 5.83 (1H, br s, H-1 of Rha″), 5.81 (1H, d, $J$ = 5.5 Hz, H-1 of Rib), 5.40 (1H, br s, H-1 of Rha′), 5.39 (1H, overlapping, H-12), 5.11 (1H, d, $J$ = 7.8 Hz, H-1 of Glc′), 4.99 (1H, d, $J$ = 7.8 Hz, H-1 of Glc‴), 4.95 (1H, d, $J$ = 7.8 Hz, H-1 of Glc), 4.83 (1H, d, $J$ = 5.9 Hz, H-1 of Ara), 3.27 (1H, dd, $J$ = 11.0, 3.7 Hz, H-3), 3.15 (1H, dd, $J$ = 13.5, 3.7 Hz, H-18), 1.68 (3H, d, $J$ = 6.1 Hz, Me-6 of Rha″), 1.57 (3H, d, $J$ = 5.5 Hz, Me-6 of Rha′), 1.53 (3H, d, $J$ = 5.9 Hz, Me-6 of Rha), 1.27 (3H, s, Me-23), 1.23 (3H, s, Me-27), 1.12 (3H, s, Me-24), 1.05 (3H, s, Me-26), 0.89 (3H, s, Me-29), 0.88

(3H, s, Me-30), 0.85 (3H, s, Me-25); $^{13}$C NMR, see Tables 1 and 3; FABMS (positive mode) *m/z* 1829 $[M + Na]^+$, 1683 $[(M + Na) - \text{deoxyhexosyl}]^+$, 1359 $[(M + Na) - \text{deoxyhexosyl} - \text{hexosyl} \times 2]^+$; FABMS (negative mode) *m/z* 1805 $[M - H]^-$, 1335 $[(M - H) - \text{deoxyhexosyl} - \text{hexosyl} \times 2]^-$; *anal.* C 52.94%, H 7.71%, calcd for $C_{82}H_{134}O_{43}\cdot 3H_2O$, C 52.90%, H 7.58%.

**Acid Hydrolysis of 5.** Compound **5** (60.2 mg) was subjected to acid hydrolysis as described for **1** to give oleanolic acid (13.6 mg) and a sugar fraction (21.6 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-arabinose, D-glucose, L-rhamnose, and D-ribose.

**Alkaline Hydrolysis of 5.** Compound **5** (20.3 mg) was treated with 6% KOH in EtOH$-H_2O$ (1:1, 4 mL) at 95 °C for 1 h under an Ar atmosphere. The mixture was neutralized by passage through an Amberlite IR-120B column and then chromatographed over silica gel eluting with $CHCl_3-MeOH-H_2O$ (7:4:1) to yield **1** (4.7 mg).

**Cell Culture Assay.** HL-60 cells, obtained from Human Science Research Resources Bank (JCRB 0085, Osaka, Japan), were maintained in RPMI 1640 medium containing heat-inactivated 10% FBS supplemented with L-glutamine, 100 units/mL penicillin, and 100 $\mu$g/mL streptomycin. The leukemia cells were washed and resuspended in the above medium to $4 \times 10^4$ cells/mL, and 196 $\mu$L of this cell suspension was placed in each well of a 96-well flat-bottom plate (Iwaki Glass, Chiba, Japan). The cells were incubated in 5% $CO_2$/air for 24 h at 37 °C. After incubation, 4 $\mu$L of EtOH$-H_2O$ (1:1) solution containing the sample was added to give the final concentrations of 0.1$-$50 $\mu$g/mL; 4 $\mu$L 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 by an MTT assay procedure. At the end of incubation, 10 $\mu$L of 5 mg/mL MTT in phosphate-buffered saline was added to every well and the plate was further incubated in 5% $CO_2$/air for 4 h at 37 °C. The plate was then centrifuged at 1500*g* for 5 min to precipitate cells and formazan. An aliquot of 150 $\mu$L of the supernatant was removed from every well, and 175 $\mu$L 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 (Spectra Classic, Tecan, Salzburg, Austria) at 550 nm. Each assay was done in triplicate, and cytotoxicity was expressed as $IC_{50}$ value, which reduced the viable cell number by 50%.

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

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NP040088K