New Bisdesmosidic Triterpene Saponins from the Roots of *Pulsatilla chinensis*

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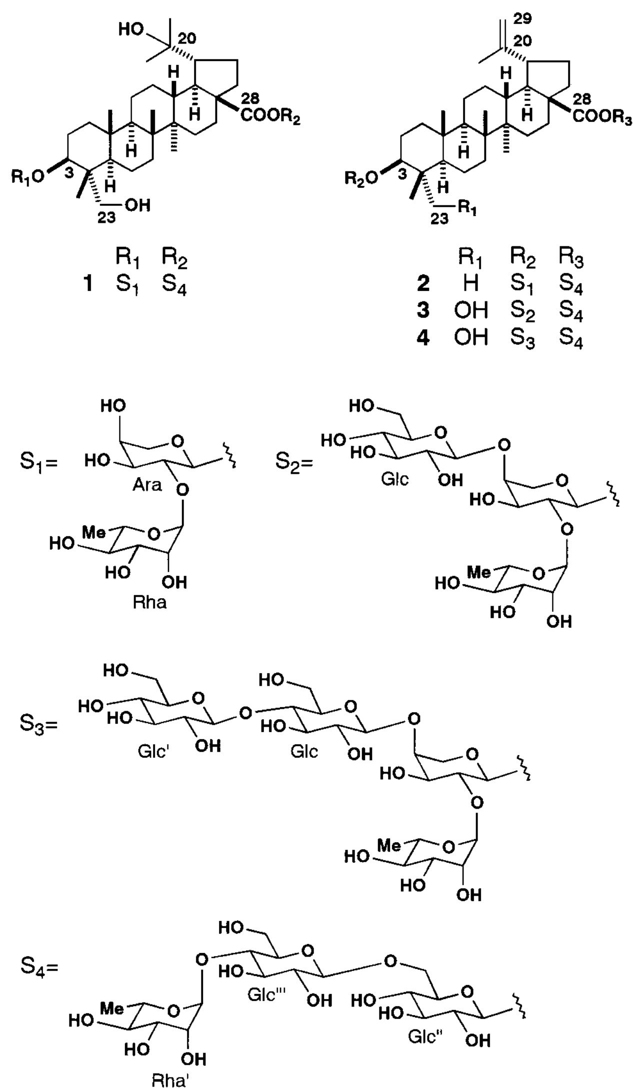
Further phytochemical analysis aimed at the triterpene saponin constituents of the roots of *Pulsatilla chinensis* has resulted in the isolation of four new bisdesmosidic triterpene saponins whose aglycons are based on the lupane skeleton (**1**−**4**), together with three known saponins (**5**−**7**). The structures of the new compounds were determined by spectroscopic analysis and acid-catalyzed hydrolysis.

The roots of *Pulsatilla chinensis* (Bunge) Regel (Ranunculaceae) have been used in traditional Chinese medicine for the treatment of intestinal amebiasis, malaria, vaginal trichomoniasis, and bacterial infections.[1] Previously, we have prepared two saponin-enriched fractions by passing an MeOH extract of *P. chinensis* roots through a polystyrene resin (Diaion HP-20) column eluted with an H_2O−MeOH gradient and then MeOH. The MeOH eluate portion, which contained relatively less polar saponins, was found to show demonstrable cytotoxic activity against cultured HL-60 human promyelocytic leukemia cells. Bioassay-guided separation of the MeOH eluate fraction yielded several monodesmosidic triterpene saponins based upon oleanolic acid and a podophyllotoxin derivative as the active components responsible for HL-60 cell cytostasis.[2] Although another saponin fraction, which was the portion eluted with a mixture of H_2O−MeOH (1:4), did not exhibit apparent cytotoxicity at a sample concentration of 10 μg/mL, we had an interest in the saponin constituents of the fraction from the viewpoint of pure phytochemistry concerning the secondary metabolites of this medicinal plant. Chromatographic separations of the H_2O−MeOH (1:4) eluate fraction have resulted in the isolation of four new bisdesmosidic triterpene saponins based on the lupane skeleton (**1**−**4**), together with three known saponins. In this paper, we report the structural determination of the new saponins on the basis of spectroscopic analysis and acid-catalyzed hydrolysis.

The MeOH extract of *P. chinensis* roots was passed through a Diaion HP-20 column. The H_2O−MeOH (1:4) eluate portion was subjected to column chromatography on Si gel and octadecylsilanized (ODS) Si gel to give compounds **1**−**7**. Compounds **5**−**7** were identified as 23-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid,[3] 3β-[(α-L-arabinopyranosyl)oxy]-23-hydroxylup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester,[4] and 23-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester,[3] respectively.



Compound **1** was obtained as an amorphous solid. Its molecular formula was derived as $C_{59}H_{98}O_{27}$ by data from the positive-ion FABMS, which showed a $[M + K]^+$ ion at *m*/*z* 1277 and a $[M + Na]^+$ ion at *m*/*z* 1261. The ^{13}C NMR spectrum, with a total of 59 carbon signals, and the results of elemental analysis were consistent with the deduced formula. The ^{1}H NMR spectrum showed signals for six triterpenoid methyl groups at δ 1.35, 1.28, 1.18, 1.02, 1.00, and 0.86, as well as signals for five anomeric protons at δ 6.31 (d, J = 8.2 Hz), 6.17 (br s), 5.80 (br s), 5.10 (d, J = 5.9 Hz), and 4.95 (d, J = 7.8 Hz). Acid hydrolysis of **1** with 0.4 M HCl in dioxane−H_2O (1:1) gave D-glucose, L-arabinose, and L-rhamnose as the carbohydrate moieties. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate, with detection being carried out by using an optical rotation (OR) detector. Comparison of the ^{1}H and ^{13}C NMR spectra of **1** with those of **7** showed their considerable structural

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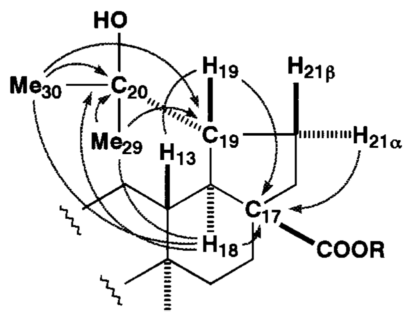

Figure 1. HMBC (arrows) and NOE (curved lines) correlations of the aglycon moiety of **1**.

similarity and confirmed that the diglycoside of *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl and the triglycoside of *O*-α-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl were attached at C-3 and C-28 of the lupan-28-oic acid derivative, respectively, as in **7**. The differences between **1** and **7** consisted only of the signals due to the E-ring part of the lupane skeleton. The deshielded three-proton singlet signal at *δ* 1.70 and a pair of exomethylene proton signals at *δ* 4.85 and 4.70 (ABq, *J* = 1.4 Hz) observed in the ^{1}H NMR spectrum of **7** were displaced by two three-proton singlets at *δ* 1.35 and 1.28 in that of **1**. The methine proton at *δ* 2.57 was shown to be coupled with both the methine proton at *δ* 1.89 and the methylene protons at *δ* 2.16 and 1.73 by the $^{1}H-^{1}H$ COSY spectrum. In the HMBC spectrum, the *δ* 2.57, 1.89, and 1.73 resonances showed long-range correlations with the quaternary carbon resonating at *δ* 59.5, to which the carboxyl group esterified with the triglycoside was linked. Thus, the signals at *δ* 1.89, 2.57, and 2.16 and 1.73 were assigned to H-18, H-19, and H_2-21, respectively. The H-19 signal was associated with the *δ* 49.7 resonance from the HMQC spectrum. In the HMBC spectrum, the methyl singlets at *δ* 1.35 and 1.28 exhibited $^{2}J_{C,H}$ correlations with the downfield-shifted quaternary carbon signal at *δ* 72.2 and $^{3}J_{C,H}$ correlations with the signal at *δ* 49.7 and were assigned to Me-29 and Me-30 (Figure 1). These data indicated that **1** is the C-20(29)-saturated derivative of **7** with the introduction of a hydroxyl group at C-20. The C-19α configuration was ascertained by NOE correlations from H-13 [*δ* 2.75 (ddd, *J* = 11.9, 11.9, 2.9 Hz)] to H-19, and from H-18 to Me-29 and Me-30 (Figure 1). Accordingly, the aglycon moiety of **1** was determined to be 3*β*,20,23-trihydroxylupan-28-oic acid, which has not been reported previously, and **1** to be 20,23-dihydroxy-3*β*-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]lupan-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

Compound **2** was shown to have the molecular formula $C_{59}H_{96}O_{25}$ on the basis of the positive-ion FABMS (*m/z* 1243 $[M + K]^+$ and 1227 $[M + Na]^+$), ^{13}C NMR spectrum, and elemental analysis. The ^{1}H NMR spectrum of **2** showed signals for six methyl groups at *δ* 1.68, 1.12, 1.07, 1.01, 1.00, and 0.74 and an exomethylene group at *δ* 4.82 and 4.68 (ABq, *J* = 1.7 Hz), which were characteristic of the lup-20(29)-en structure, as well as signals for five anomeric protons. Analysis of the ^{13}C NMR spectrum of **2** and comparison with that of **7** revealed that the diglycoside structure attached at C-3 and the triglycoside at C-28 were identical to those of **7**, but differed slightly from **7** in terms of the aglycon structure. Acid hydrolysis of **2** with 0.4 M HCl liberated a known triterpenoid sapogenin identified as 3*β*-hydroxylup-20(29)-en-28-oic acid,[5] and D-glucose, L-arabinose, and L-rhamnose. Thus, **2** was shown to be a new combination of a known triterpenoid sapogenin and sugars, and its structure was formulated as 3*β*-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]lup-20-(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

Compound **3** was analyzed for $C_{65}H_{106}O_{31}$ by combined FABMS, ^{13}C NMR, and elemental analysis. The deduced molecular formula was higher by $C_6H_{10}O_5$ than that of **7**, and the ^{1}H NMR spectrum showed signals for six anomeric protons at *δ* 6.31 (d, *J* = 8.2 Hz), 6.18 (br s), 5.81 (br s), 5.08 (d, *J* = 7.9 Hz), 4.98 (d, *J* = 6.5 Hz), and 4.93 (d, *J* = 7.8 Hz), along with signals for five triterpenoid methyl protons at *δ* 1.67, 1.11, 1.03, 0.94, and 0.84 (each s) and exomethylene protons at *δ* 4.82 and 4.67 (each br s). Acid hydrolysis of **3** with 0.4 M HCl resulted in the production of 3*β*,23-dihydroxylup-20(29)-en-28-oic acid,[6] and D-glucose, L-arabinose, and L-rhamnose as the carbohydrate moieties. On comparison of the ^{13}C NMR spectrum of **3** with that of **7**, an additional six signals corresponding to a terminal *β*-D-glucopyranosyl moiety appeared at *δ* 106.5 (CH), 75.4 (CH), 78.3 (CH), 71.1 (CH), 78.6 (CH), and 62.3 (CH_2), and the signals due to C-4 of the arabinose moiety and its neighboring carbons varied, while all other signals remained almost unaffected. In the HMBC spectrum, correlation peaks from the anomeric proton of the glucose at *δ* 5.08 to C-4 of the arabinose at *δ* 80.0 and from the anomeric proton of the arabinose at *δ* 4.98 to C-3 of the aglycon at *δ* 81.0 confirmed that the additional glucose was linked to C-4 of the arabinose, which, in turn, was attached to C-3 of the aglycon. An HMBC correlation between the signals of the anomeric proton of the rhamnose at *δ* 6.18 and the C-2 carbon of the arabinose at *δ* 76.2 was also noted. The structure of **3** was thus formulated as 23-hydroxy-3*β*-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

Compound **4** was deduced as $C_{71}H_{116}O_{36}$ from its FABMS, ^{13}C NMR spectal, and elemental analysis data. The ^{1}H and ^{13}C NMR spectra of **4** allowed the identification of its aglycon as 3*β*,23-dihydroxylup-20(29)-en-28-oic acid, and the ^{1}H NMR spectrum displayed seven anomeric proton signals at *δ* 6.32 (d, *J* = 8.1 Hz), 6.22 (br s), 5.82 (br s), 5.16 (d, *J* = 7.9 Hz), 5.05 (d, *J* = 7.9 Hz), 4.98 (d, *J* = 6.6 Hz), and 4.93 (d, *J* = 7.8 Hz). Acid hydrolysis of **4** with 0.4 M HCl gave 3*β*,23-dihydroxylup-20(29)-en-28-oic acid, D-glucose, L-arabinose, and L-rhamnose. These results and comparison of the ^{13}C NMR spectrum of **4** with that of **3** suggested that **4** structurally corresponded to **3** with one more *β*-D-glucose unit present. However, the presence of seven sugar units in **4** generated severe overlapping of the glycoside protons, which caused difficulties in assigning the ^{13}C NMR shifts by the $^{1}H-^{1}H$ COSY and HMQC spectra. The HSQC-TOCSY spectrum measured by varying the mixing time was necessary for the unequivocal ^{13}C NMR assignments, because it correlated each anomeric proton and the easily distinguished methyl doublets of the rhamnoses with their respective skeleton carbon atoms. Although relayed cross-peaks terminated at C-4 of the arabinose, C-5 of the arabinose was assigned by the concerted use of $^{1}H-^{1}H$ COSY and HMQC spectra. Comparison of the ^{13}C NMR signals thus assigned for the sugar moieties of **4** with those of **3**, taking into account the known effects of *O*-glycosylation, indicated that the sugar chain made up of three monosaccharides attached at C-28 was identical to that of **3** and that the sugar linked to C-3 included a *β*-D-glucopyranosyl unit glycosylated at C-4, as well as a terminal *β*-D-glucopyranosyl unit, a terminal α-L-rhamnopyranosyl unit, and an intermediate α-L-arabinopy-

ranosyl unit glycosylated at C-2 and C-4. The anomeric proton at δ 5.16 attributable to the terminal glucose unit showed a long-range correlation with C-4 of the substituted glucose at δ 81.2, whose anomeric proton at δ 5.05, in turn, was correlated to C-4 of the arabinose at δ 80.4. HMBC correlations between the signals of the anomeric proton of the rhamnose at δ 6.22 and the C-2 carbon of the arabinose at δ 76.2 and between the anomeric proton of the arabinose at δ 4.98 and the C-3 of the aglycon at δ 81.1, were also detected. Accordingly, the structure of **4** was defined as 23-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Compounds **1**−**7** did not show any apparent cytotoxic activity against HL-60 cells at a sample concentration of 10 μg/mL, but 23-hydroxy-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid, which has been isolated from the MeOH eluate fraction of *P. chinensis* roots and differs from **5** only by the aglycon E-ring partial structure, was cytotoxic to HL-60 cells with an IC_{50} value of 7.1 μg/mL.[2] This fact suggests that the structures of the aglycon portions, as well as the sugar sequences,[2] contribute to the exhibition of cytotoxic activity of triterpene saponins from *P. chinensis*.

Table 1. ^{13}C NMR Data for the Aglycon Moiety of **1**−**4**[a]

carbon	**1**	**2**	**3**	**4**
1	39.2	39.0	39.1	39.2
2	26.3	26.6	26.4	26.4
3	81.0	88.7	81.0	81.1
4	43.5	39.4	43.5	43.5
5	47.7	55.9	47.8	47.8
6	18.1	18.4	18.0	18.0
7	34.6	34.4	34.1	34.2
8	41.6	41.0	41.0	41.1
9	51.0	50.7	50.8	50.9
10	36.8	37.0	36.9	36.9
11	21.9	20.9	21.0	21.1
12	29.6	25.9	25.9	26.0
13	38.5	38.2	38.2	38.3
14	43.6	42.6	42.7	42.7
15	30.6	30.0	30.0	30.1
16	32.4	32.1	32.1	32.2
17	59.5	56.8	56.9	56.9
18	49.2	49.6	49.7	49.7
19	49.7	47.2	47.3	47.3
20	72.2	150.7	150.7	150.8
21	29.0	30.7	30.7	30.8
22	36.6	36.7	36.8	36.8
23	63.8	27.9	63.7	63.7
24	13.6	16.6	13.7	13.7
25	17.0	16.3	16.8	16.9
26	16.8	16.2	16.3	16.3
27	15.2	14.7	14.8	14.8
28	175.4	174.8	174.9	174.9
29	27.0	109.9	110.0	110.0
30	31.4	19.3	19.3	19.3

[a] Spectra were measured in C_5D_5N.

Experimental Section

General Experimental Procedures. The instruments and experimental conditions, as well as the plant material used, were the same as described in a previous paper.[2]

Extraction and Isolation. Solvent extraction was performed as published previously.[2] The resultant MeOH extract of *P. chinensis* roots was passed through a Diaion HP-20 column, and the H_2O−MeOH (1:4) eluate portion[2] was subjected to column chromatography on Si gel, eluting with stepwise gradient mixtures of $CHCl_3$−MeOH−H_2O (60:10:1; 20:10:1; 7:4:1) and finally with MeOH alone, to give eight fractions (I−VIII). Fraction II was chromatographed on ODS Si gel eluting with MeCN−H_2O (1:1; 5:8) to yield **5** (14.1 mg). Fraction III was purified by passing it over a Si gel column eluting with $CHCl_3$−MeOH−H_2O (20:10:1; 7:4:1) and an ODS Si gel column with MeCN−H_2O (1:2) to afford **2** (27.5 mg) and **6** (79.4 mg). Fraction IV was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (7:4:1) to give **7** (17.4 g). Fraction V was subjected to column chromatography on Si gel eluting with $CHCl_3$−MeOH−H_2O (7:4:1) and ODS Si gel with MeCN−H_2O (1:1) to yield **1** (29.8 mg). Fraction VI was refined using Si gel column chromatography eluting with $CHCl_3$−MeOH−H_2O (20:10:1; 7:4:1) and ODS Si gel column chromatography with MeCN−H_2O (5:8; 1:3) to furnish **3** (1.28 g). Compound **4** (25.8 mg) was isolated from fraction VII by subjecting it to Si gel column chromatography eluting with $CHCl_3$−MeOH−H_2O (7:4:1) and ODS Si gel column chromatography with MeOH−H_2O (8:5).

Compound 1: amorphous solid; $[\alpha]_D^{26}$ −48.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3388 (OH), 2939 (CH), 1731 (C=O), 1058 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.31 (1H, d, *J* = 8.2 Hz, H-1 of Glc″), 6.17 (1H, br s, H-1 of Rha), 5.80 (1H, br s, H-1 of Rha′), 5.10 (1H, d, *J* = 5.9 Hz, H-1 of Ara), 4.95 (1H, d, *J* = 7.8 Hz, H-1 of Glc‴), 4.20 (1H, m, H-3), 4.09 and 3.71 (each 1H, ABq, *J* = 11.7 Hz, H_2-23), 2.75 (1H, ddd, *J* = 11.9, 11.9, 2.9 Hz, H-13), 2.57 (1H, ddd, *J* = 9.0, 9.0, 1.6 Hz, H-19), 2.16 (1H, m, H-21β), 1.89 (1H, dd, *J* = 11.9, 9.0 Hz, H-18), 1.73 (1H, m, H-21α), 1.66 (3H, d, *J* = 6.2 Hz, Me-6 of Rha′), 1.60 (3H, d, *J* = 6.2 Hz, Me-6 of Rha), 1.35 (3H, s, Me-30), 1.28 (3H, s, Me-29), 1.18 (3H, s, Me-26), 1.02 (3H, s, Me-27), 1.00 (3H, s, Me-24), 0.86 (3H, s, Me-25); FABMS (positive mode) *m/z* 1277 $[M + K]^+$, 1261 $[M + Na]^+$; *anal.* C 54.41%, H 7.80%, calcd for $C_{59}H_{98}O_{27}\cdot 7/2H_2O$, C 54.41%, H 8.13%.

Acid Hydrolysis of 1. A solution of **1** (13.5 mg) in 0.4 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 90 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on Si gel using a discontinuous gradient of $CHCl_3$−MeOH (99:1 to 1:1) to give a sugar fraction (5.6 mg). The sugar fraction was dissolved in H_2O and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA), which was then analyzed by HPLC under the following conditions: column, Kaseisorb LC NH_2 Super (4.6 mm i.d. × 250 mm, 5 μm, Tokyo-Kasei, Japan); solvent, MeCN−H_2O (3:1); flow rate, 0.4 mL/min; detection, OR. Identification of D-glucose, L-arabinose, and L-rhamnose present in the sugar fraction was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R (min): 10.86 (L-rhamnose, negative optical rotation); 11.98 (L-arabinose, positive optical rotation); 13.46 (D-glucose, positive optical rotation).

Compound 2: amorphous solid; $[\alpha]_D^{26}$ −34.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3387 (OH), 2940 (CH), 1742 (C=O), 1061 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.29 (1H, d, *J* = 8.2 Hz, H-1 of Glc″), 6.06 (1H, br s, H-1 of Rha), 5.79 (1H, br s, H-1 of Rha′), 4.92 (1H, d, *J* = 7.8 Hz, H-1 of Glc‴), 4.86 (1H, d, *J* = 5.3 Hz, H-1 of Ara), 4.82 and 4.68 (each 1H, ABq, *J* = 1.7 Hz, H_2-29), 3.21 (1H, dd, *J* = 11.6, 4.1 Hz, H-3), 1.68 (3H, s, Me-30), 1.64 (3H, d, *J* = 6.2 Hz, Me-6 of Rha′), 1.58 (3H, d, *J* = 6.2 Hz, Me-6 of Rha), 1.12 (3H, s, Me-23), 1.07 (3H, s, Me-26), 1.01 (3H, s, Me-27), 1.00 (3H, s, Me-24), 0.74 (3H, s, Me-25); FABMS (positive mode) *m/z* 1243 $[M + K]^+$, 1227 $[M + Na]^+$, 757 $[M + Na - \text{rhamnosyl} - \text{glucosyl} \times 2]^+$; *anal.* C 57.73%, H 8.20%, calcd for $C_{59}H_{96}O_{25}\cdot H_2O$, C 57.92%, H 8.07%.

Acid Hydrolysis of 2. Compound **2** (14.2 mg) was subjected to acid hydrolysis as described for **1**, and the crude hydrolysate was chromatographed on Si gel, eluting initially with $CHCl_3$−Me_2CO (4:1) and then with $CHCl_3$−MeOH (1:1), to furnish 3β-hydroxylup-20(29)-en-28-oic acid (3.1 mg) and a sugar fraction (6.2 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1** showed the presence of D-glucose, L-arabinose, and L-rhamnose.

Compound 3: amorphous solid; $[\alpha]_D^{26}$ −38.4° (*c* 0.10, MeOH); IR (film) ν_{max} 3387 (OH), 2939 (CH), 1733 (C=O), 1070 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.31 (1H, d, *J* = 8.2 Hz, H-1 of Glc″), 6.18 (1H, br s, H-1 of Rha), 5.81 (1H, br s, H-1 of Rha′), 5.08

Table 2. ^{13}C NMR Data for the Sugar Moieties of **1**−**4**[a]

carbon	1	2	3	4
Ara 1	104.1	104.7	104.2	104.3
2	75.7	75.9	76.2	76.2
3	74.4	73.6	74.5	74.8
4	69.1	68.5	80.0	80.4
5	65.3	64.5	65.1	65.2
Rha 1	101.5	101.6	101.6	101.6
2	72.2	72.2	72.1	72.1
3	72.4	72.4	72.4	72.4
4	74.0	73.8	74.0	74.0
5	69.6	69.7	69.6	69.6
6	18.4	18.4	18.5	18.5
Glc 1			106.5	106.1
2			75.4	74.9
3			78.3	76.6
4			71.1	81.2
5			78.6	76.6
6			62.3	61.7
Glc′ 1				104.9
2				74.7
3				78.2
4				71.5
5				78.3
6				62.4
Glc″ 1	95.1	95.1	95.1	95.2
2	73.9	73.9	74.0	74.0
3	78.6	78.4	78.5	78.6
4	70.7	70.6	70.7	70.8
5	77.9	77.8	77.9	77.9
6	69.3	69.2	69.3	69.4
Glc‴ 1	104.9	104.8	104.9	105.0
2	75.2	75.2	75.2	75.2
3	76.4	76.3	76.3	76.4
4	78.2	78.0	78.1	78.2
5	77.0	76.9	77.0	77.0
6	61.2	61.1	61.1	61.2
Rha′1	102.6	102.4	102.5	102.6
2	72.4	72.3	72.4	72.5
3	72.6	72.6	72.6	72.7
4	73.9	73.9	73.9	73.9
5	70.2	70.1	70.2	70.2
6	18.4	18.4	18.4	18.4

[a] Spectra were measured in C_5D_5N.

(1H, d, J = 7.9 Hz, H-1 of Glc), 4.98 (1H, d, J = 6.5 Hz, H-1 of Ara), 4.93 (1H, d, J = 7.8 Hz, H-1 of Glc‴), 4.82 and 4.67 (each 1H, br s, H_2-29), 4.18 (1H, m, H-3), 4.10 and 3.71 (each 1H, ABq, J = 10.5 Hz, H_2-23), 1.67 (3H, s, Me-30), 1.66 (3H, d, J = 6.2 Hz, Me-6 of Rha′), 1.61 (3H, d, J = 6.1 Hz, Me-6 of Rha), 1.11 (3H, s, Me-26), 1.03 (3H, s, Me-24), 0.94 (3H, s, Me-27), 0.84 (3H, s, Me-25); FABMS (positive mode) m/z 1389 [M + Li]$^+$, 919 [M + Li − rhamnosyl − glucosyl × 2]$^+$; *anal.* C 55.38%, H 7.87%, calcd for $C_{65}H_{106}O_{31}\cdot 3/2H_2O$, C 55.35%, H 7.79%.

Acid Hydrolysis of 3. Compound **3** (50 mg) was subjected to acid hydrolysis as described for **2** to give 3β,23-dihydroxylup-20(29)-en-28-oic acid (9.1 mg) and a sugar fraction (24.4 mg). HPLC analysis of the sugar fraction showed the presence of D-glucose, L-arabinose, and L-rhamnose.

Compound 4: amorphous solid; $[\alpha]_D^{26}$ −38.0° (c 0.10, MeOH); IR (film) ν_{max} 3387 (OH), 2937 (CH), 1733 (C=O), 1068 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 6.32 (1H, d, J = 8.1 Hz, H-1 of Glc″), 6.22 (1H, br s, H-1 of Rha), 5.82 (1H, br s, H-1 of Rha′), 5.16 (1H, d, J = 7.9 Hz, H-1 of Glc′), 5.05 (1H, d, J = 7.9 Hz, H-1 of Glc), 4.98 (1H, d, J = 6.6 Hz, H-1 of Ara), 4.93 (1H, d, J = 7.8 Hz, H-1 of Glc‴), 4.83 and 4.68 (each 1H, br s, H_2-29), 4.19 (1H, m, H-3), 4.11 and 3.71 (each 1H, ABq, J = 10.4 Hz, H_2-23), 1.68 (3H, s, Me-30), 1.68 (3H, d, J = 6.2 Hz, Me-6 of Rha′), 1.62 (3H, d, J = 6.1 Hz, Me-6 of Rha), 1.12 (3H, s, Me-26), 1.05 (3H, s, Me-24), 0.95 (3H, s, Me-27), 0.86 (3H, s, Me-25); FABMS (positive mode) m/z 1551 [M + Li]$^+$, 1081 [M + Li − rhamnosyl − glucosyl × 2]$^+$; *anal.* C 53.04%, H 7.75%, calcd for $C_{71}H_{116}O_{36}\cdot 4H_2O$, C 52.71%, H 7.73%.

Acid Hydrolysis of 4. Compound **4** (13.6 mg) was subjected to acid hydrolysis as described for **2** to give 3β,23-dihydroxylup-20(29)-en-28-oic acid (3.2 mg) and a sugar fraction (6.8 mg). HPLC analysis of the sugar fraction showed the presence of D-glucose, L-arabinose, and L-rhamnose.

HL-60 Cell Culture Assay. The cell growth was measured with an MTT reduction assay procedure as described in a previous paper.[2]

References and Notes

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