Triterpenoids from the Resin of *Styrax tonkinensis* and Their Antiproliferative and Differentiation Effects in Human Leukemia HL-60 Cells

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Four new triterpenoids, 6β-hydroxy-3-oxo-11α,12α-epoxyolean-28,13β-olide (**1**), 3β,6β-dihydroxy-11α,12α-epoxyolean-28,13β-olide (**2**), 3β,6β-dihydroxy-11-oxo-olean-12-en-28-oic acid (**3**), and 3β-hydroxy-12-oxo-13Hα-olean-28,19β-olide (**4**), and five known triterpenes, 19α-hydroxy-3-oxo-olean-12-en-28-oic acid (**5**), 6β-hydroxy-3-oxo-olean-12-en-28-oic acid (**6**), sumaresinolic acid (**7**), siaresinolic acid (**8**), and oleanolic acid (**9**), were isolated from the resin of *Styrax tonkinensis*. The structures of these triterpenoids were determined by physicochemical and spectroscopic methods. The configuration of compound **4** was confirmed by X-ray crystallographic analysis. All these triterpenoids inhibited HL-60 cell growth with IG_{50} values ranging from 8.9 to 99.4 μM. Oleanolic acid (**9**) was the most effective antiproliferative agent, with an IG_{50} value of 8.9 μM. While 3β,6β-dihydroxy-11-oxo-olean-12-en-28-oic acid (**3**) exhibited the least effective growth inhibition among these triterpenoids, it induced HL-60 cells to undergo differentiation as measured by an NBT reduction assay.

Styrax tonkinensis (Pier.) Craib (Styracaceae) is an tree distributed in several regions of Southeast Asia. Its resin has been used as an expectorant in China. One triterpenoid, siaresinolic acid, and some aromatic compounds, benzoic acid, vanillin, coniferyl benzoate, and cinnamyl benzoate, have been reported from the resin of *S. tonkinensis*.[1] Recently, we have found two new aromatics, *trans*-[tetrahydro-2-(4-hydroxy-3-methoxyphenyl)-5-oxofuran-3-yl]-methyl benzoate and 3-(4-hydroxyl-3-methoxyphenyl)-2-oxopropyl benzoate, and one new natural product, 4-[(*E*)-3-ethoxyprop-1-enyl]-2-methoxyphenol.[2] We now report the identification of four new triterpenoids, 6β-hydroxy-3-oxo-11α,12α-epoxyolean-28,13β-olide (**1**), 3β,6β-dihydroxy-11α,12α-epoxyolean-28,13β-olide (**2**), 3β,6β-dihydroxy-11-oxo-olean-12-en-28-oic acid (**3**), and 3β-hydroxy-12-oxo-13Hα-olean-28,19β-olide (**4**), along with five known triterpenoids, 19α-hydroxy-3-oxo-olean-12-en-28-oic acid (**5**),[3] 6β-hydroxyl-3-oxo-olean-12-en-28-oic acid (**6**),[4] sumaresinolic acid (**7**), siaresinolic acid (**8**),[5] and oleanolic acid (**9**).[6]

Triterpenoids and their saponins have been found in many plants.[7] Some triterpenoids including oleanoic acid were found to have cytotoxic and differentiation effects in leukemia cells.[8] In this study, the antiproliferative and differentiation effects of these triterpenoids in human HL-60 leukemia cells were determined.

Compound **1** was obtained as colorless needles, and its molecular formula was determined as $C_{30}H_{44}O_5$ on the basis of its HREIMS spectrum (*m*/*z* 484.3193 $[M]^+$). The ^1H NMR spectrum showed seven methyl singlets (Table 1), and the ^{13}C NMR spectrum revealed 30 carbon signals, which were sorted by DEPT experiment as seven methyls, eight methylenes, six methines, and nine quaternary carbons, of which three oxygenated methines, one oxygenated quaternary carbon, and two carbonyl groups were suggested on the basis of the chemical shifts. The IR absorptions of compound **1** at 3568, 1755, 1708, 930, and 872 cm^{-1} revealed the presence of hydroxy, γ-lactone, ketone carbonyl, and epoxide groups. On the basis of the above data compound **1** was an oleanane-type triterpene. The ^1H and ^{13}C NMR data were assigned by the $^1H-^1H$ COSY, HSQC, and HMBC spectra (Tables 1 and 2). The resonances at δ 52.5, 57.0 were characteristic of the 11α,12α-epoxide moiety, and the signals at δ 179.2 (C-28), 87.2 (C-13) indicated the presence of the 28,13β-lactone unit.[9] In addition,

Table 1. ^1H NMR Data of Compounds **1**−**4**

	1	**2**	**3**	**4**
H	δ_H (*J*, Hz) $CDCl_3$ (600 MHz)	δ_H (*J*, Hz) pyridine-d_5 (600 MHz)	δ_H (*J*, Hz) pyridine-d_5 (600 MHz)	δ_H (*J*, Hz) pyridine-d_5 (600 MHz)
1a	2.21 m	1.67 m	1.35 m	1.43 m
1b	1.49 m	1.59 m	1.25 m	0.95 m
2a	2.93 td (14.6, 6.0)	2.11 m	2.21 m	1.78 m
2b	2.31 m	1.93 m	2.00 m	1.23 m
3		3.46 dd (12.0, 3.9)	3.51 dd (10.8, 3.6)	3.41 t (7.8)
5	1.10 brs	0.91 brs	1.02 brs	0.83 m
6a	4.53 brs	4.79 brs	4.85 brs	1.52 m
6b				1.33 m
7a	1.53 m	1.89 m	1.95 m	1.33 m
7b	1.18 m	1.26 m	1.84 m	1.25 m
9	1.64 m	1.85 m	2.74 s	2.06 dd (9.5, 9.5)
11a	3.16 brd (3.6)	3.29 m		2.39 m
11b				2.44 m
12	3.10 d (3.6)	3.30 m	6.11 s	
13				2.77 d (12.6)
15a	1.75 td (13.2, 5.6)	1.80 m	1.82 m	2.32 m
15b	1.34 m	1.04 m	1.21 m	1.68 m
16a	2.13 td (13.2, 5.6)	2.12 m	2.12 m	1.42 dt (14.4, 4.8)
16b	1.08 m	1.25 m	1.97 m	0.99 td (14.4, 5.8)
18	2.32 m	2.52 brd (13.8)	3.37 m	2.54 dd (12.6, 4.8)
19a	1.84 t (13.6)	1.95 m	1.75 m	5.03 d (4.8)
19b	1.63 m	1.73 m	1.23 m	
21a	1.33 m	1.31 m	1.95 m	1.51 m
21b		1.12 m	1.84 m	1.24 m
22a	1.65 m	1.73 m	1.43 m	1.93 td (13.8, 5.6)
22b		1.67 m		1.35 m
23	1.17 s	1.38 s	1.44 s	1.19 s
24	1.45 s	1.69 s	1.75 s	0.98 s
25	1.64 s	1.61 s	2.01 s	0.85 s
26	1.48 s	1.80 s	1.82 s	0.80 s
27	1.04 s	1.19 s	1.44 s	1.28 s
29	0.93 s	0.84 s	0.92 s	0.95 s
30	1.00 s	0.76 s	0.91 s	0.92 s

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Table 2. ^{13}C NMR Data of Compounds **1**−**4**, **7**, and **8**

C	**1** $CDCl_3$ (75 MHz)	**2** pyridine-d_5 (75 MHz)	**3** pyridine-d_5 (75 MHz)	**4** pyridine-d_5 (75 MHz)	**7** $CDCl_3$ (75 MHz)	**8** pyridine-d_5 (75 MHz)
1	41.3	39.8	41.8	38.5	38.8	41.7
2	34.3	28.0	28.3	27.8	34.1	33.9
3	215.3	78.6	78.5	77.9	217.4	216.4
4	49.1	40.6	40.9	39.4	47.5	47.4
5	56.3	56.1	56.0	55.6	55.4	57.7
6	69.1	67.5	66.6	18.4	19.6	68.9
7	39.5	40.8	41.1	34.8	32.0	42.6
8	40.8	41.2	44.8	41.7	39.6	40.0
9	50.9	52.0	62.8	49.8	47.1	48.7
10	35.9	36.6	37.8	38.3	37.0	37.7
11	52.5	53.1	200.1	38.5	23.7	24.5
12	57.0	57.5	128.6	214.9	124.9	123.4
13	87.2	87.7	169.2	54.4	142.7	145.0
14	40.8	41.3	44.5	44.8	41.3	43.5
15	26.9	27.1	28.5	26.1	27.4	29.0
16	21.2	21.7	23.4	26.0	23.7	24.7
17	43.8	44.1	46.2	42.0	45.3	50.0
18	49.6	49.9	42.4	45.8	43.4	42.9
19	37.8	38.2	44.7	87.2	81.5	47.2
20	31.5	31.5	30.9	32.4	34.6	31.7
21	33.9	34.5	32.3	33.6	27.9	35.5
22	26.7	27.7	34.0	26.8	32.4	35.0
23	23.5	28.0	28.5	28.6	26.2	30.0
24	24.5	17.6	18.0	39.4	21.4	24.8
25	17.8	19.0	18.3	15.9	14.7	17.0
26	20.9	21.3	20.1	17.7	17.0	19.5
27	19.1	19.1	23.8	25.8	24.9	26.5
28	179.2	179.0	179.7	180.1	184.5	180.9
29	33.2	33.1	32.9	30.5	27.9	34.0
30	23.5	23.5	23.4	22.7	24.4	24.5

the significant peak at *m*/*z* 249 in the EIMS spectrum of compound **1** confirmed the existence of an 11α,12α-epoxy moiety of the oleanane series.[9]

In the HMBC spectrum, the long-range correlation between the methyl protons at δ 1.17 (H-23), 1.45 (H-24) and the ketone carbonyl at δ 215.3 implies that the carbonyl group is located at C-3. The location of a hydroxy group at C-6 was determined by the correlation between H-6 and H-5 in the ^{1}H−^{1}H COSY spectrum. The H-5α and H-6 protons as recorded in the ^{1}H NMR spectrum at 600 MHz resonated as broad singlets at δ 1.10 and 4.53, respectively, indicating their axial−equatorial relationship. Thus, the β-orientation of the C-6 hydroxyl group was defined.[10] The 11α,12α-epoxide configuration is preferred due to the β-configuration of the 28,13β-lactone moiety.[11] On the basis of the above spectroscopic data and comparison with structurally similar liquidambaric lactones,[11] compound **1** was defined as 6β-hydroxy-3-oxo-11α,12α-epoxyolean-28,13β-olide (Figure 1).

Compound **2** was obtained as a white amorphous powder, and its molecular formula was determined as $C_{30}H_{46}O_5$ on the basis of its HREIMS spectrum (*m*/*z* 486.3337 $[M]^+$). The ^{1}H NMR spectrum showed seven methyl singlets, and the ^{13}C NMR spectrum gave 30 carbon signals, which were sorted by DEPT experiment as seven methyls, eight methylenes, three methines, six quaternary carbons, four oxygenated methines, one oxygenated quaternary carbon, and one carbonyl group.

The ^{1}H and ^{13}C NMR spectra of compound **2** are similar to those of compound **1**, including resonances at δ 53.1, 57.5 characteristic of the 11α,12α-epoxide moiety and resonances at δ 179.0 (C-28), 87.7 (C-13) typical of the 28,13β-lactone unit. Differences in the ^{13}C NMR spectra of compounds **1** and **2** are the appearance of a signal at δ 78.6 in the spectrum of **2** replacing the signal at δ 215.3 for the C-3 ketone in compound **1**, suggesting a 3-hydroxyl group in compound **2**. The β-orientation of this hydroxyl group was indicated by the chemical shift of C-5 at δ 56.1.[6] Thus, on the basis of these ^{13}C NMR data (Table 2), compound **2** was defined as 3β,6β-dihydroxy-11α,12α-epoxyolean-28,13β-olide (Figure 1).

Compound **3** was obtained as a white amorphous powder. The molecular formula was determined as $C_{30}H_{46}O_5$ on the basis of its HRFABMS spectrum (*m*/*z* 487.3423 $[M + H]^+$). The ^{1}H NMR spectrum showed seven methyl singlets, and the ^{13}C NMR spectrum revealed 30 carbon signals, which were sorted as seven methyls, eight methylenes, six methines, and nine quaternary carbons by DEPT experiments. The IR spectrum of compound **3** indicated the presence of hydroxyl (3446 cm^{-1}), carbonyl (1651 cm^{-1}), and carboxylic (1700 cm^{-1}) groups. The above data implied that compound **3** was an oleanane-type triterpene. The ^{1}H and ^{13}C NMR data were assigned by the ^{1}H−^{1}H COSY, HSQC, and HMBC spectra (Tables 1 and 2). Signals of an α,β-unsaturated carbonyl at δ 128.6, 169.2, 200.1 were present in the ^{13}C NMR spectrum. Compound **3** was found to be identical with 3β-hydroxyl-11-oxo-olean-12-en-28-oic acid[12] in the ^{1}H and ^{13}C NMR spectra except for the presence of an oxygen-bearing signal at δ 4.85 in compound **3**, which had a correlation with H-5 in the ^{1}H−^{1}H COSY spectrum, indicating a hydroxyl group at C-6. The orientation of the 6-hydroxyl group was determined as β in view of the broad H-5 and H-6 singlets (Table 1). On the basis of these, compound **3** was identified as 3β,6β-dihydroxy-11-oxo-olean-12-en-28-oic acid (Figure 1).

Compound **4** was obtained as colorless needles. Its molecular formula was determined as $C_{30}H_{46}O_4$ on the basis of its HREIMS spectrum (*m*/*z* 470.3391 $[M]^+$). This compound was suggested as an oleanane-type triterpene on the basis of ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2). The signals at δ 180.1 (C-28), 87.2 (C-19) indicated the presence of a 28,19β-lactone unit. This compound showed IR absorptions of hydroxyl, γ-lactone, and ketone carbonyl groups at 3472, 1784, and 1685 cm^{-1}, respectively.

In the HMBC spectrum, the long-range correlation between the methyl protons at δ 1.19 (H-23), 0.98 (H-24) and the oxygenated methine at δ 77.9 suggested a hydroxyl group located at C-3. The β-orientation of this hydroxyl group was confirmed by the chemical shift of C-5.[6] The carbon signal resonating at δ 87.2 was assigned to C-19 due to the long-range correlation between the methyl protons at δ 0.95 (C-29), 0.92 (C-30) and the oxygenated methine. The correlation between the protons at δ 5.03 (H-19) and δ 2.54 (H-18) revealed by ^{1}H−^{1}H COSY indicated that C-19 was linked to an oxygen atom. Furthermore, the long-range correlation between the proton at δ 2.77 (H-13, d, J = 12.6 Hz) and the ketone carbonyl group at δ 214.9 confirmed a ketone carbonyl group located at C-12. In the NOESY experiment, CH_3-27 at δ 1.28 showed correlations with H-9 at δ 2.06 (1H, dd, J = 9.5 Hz, 9.5 Hz) and H-13 at δ 2.77 (1H, d, J = 12.6 Hz), which indicated that H-13 was α-oriented and that the C/D ring was *cis*-fused, which is rarely observed in oleanane-type triterpenes. The J_{H13-18} value was 12.6 Hz, which suggested a β-oriented H-18. Thus, compound **4** was identified as 3β-hydroxy-12-oxo-13Hα-olean-28,19β-olide (Figure 1). The configuration of compound **4** was confirmed by X-ray crystallographic analysis, which showed that the C and D rings were in boat conformations (Figure 2).

By testing in human leukemia HL-60 cells, it was found that all these triterpenoids inhibited cell growth with different activity. Compound **9** was the most potent, with an IG_{50} of 8.9 μM, while compound **3** was the least effective growth inhibitor, with an IG_{50} of 99.4 μM. The IG_{50}'s of these triterpenoids are listed in Table 3. Although the triterpenoids inhibited cell growth, the cytotoxicity (based on trypan blue staining) was observed only in cells treated with compounds **2**, **4**, **7**, and **9** at double IG_{50} concentrations (loss of more than 20% viable cells). The data suggest that the triterpenoids are antiproliferative but not cytotoxic to HL-60 cells. Since it has been shown that some triterpenoids have differentiation effects,[8] the cell differentiation ability of these triterpenoids was measured in HL-60 cells using the NBT reduction assay. All the triterpenoids except compound **3**, at nontoxic concentrations, did not induce differentiation or had only a weak effect (<10% NBT-positive cells). The cell growth inhibition, cytotoxicity, and differentiation induction of compounds **3** and **9** were compared after

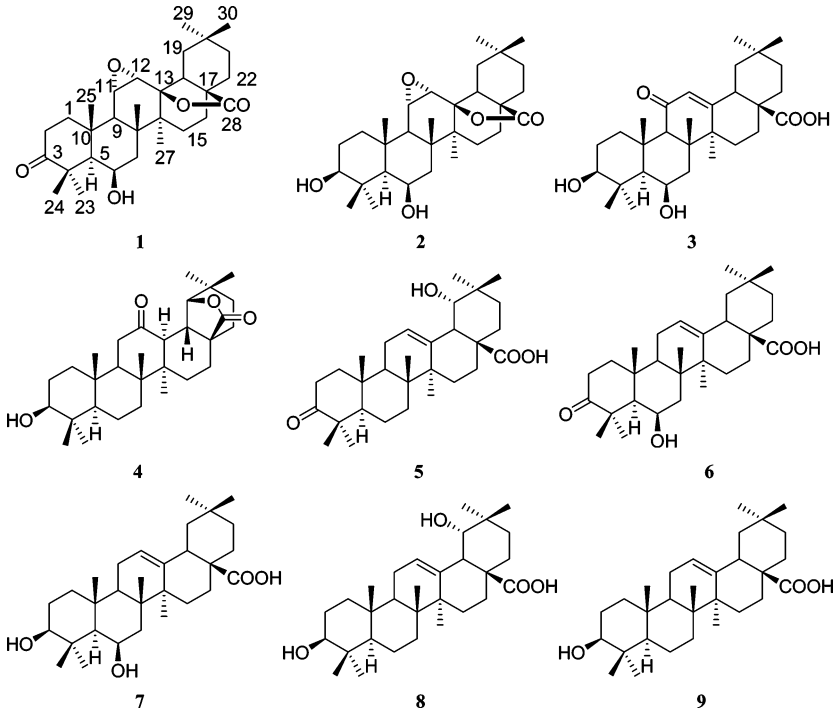

Figure 1. Structures of triterpenoids from the resin of *Styrax tonkinensis*.

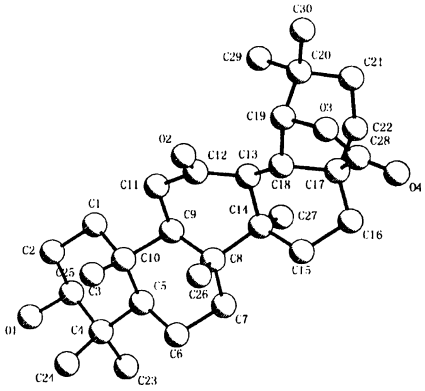

Figure 2. X-ray diffraction structure of compound **4**.

Table 3. IG_{50} Values of Triterpenoids that Inhibit HL-60 Cell Growth[a]

compound	$IG_{50} \pm$ SE (μM)
1	41.8 ± 3.7
2	27.5 ± 7.9
3	99.4 ± 12.8
4	51.2 ± 1.4
5	41.0 ± 3.8
6	14.2 ± 4.9
7	30.2 ± 2.1
8	29.0 ± 3.1
9	8.9 ± 0.8

[a] HL-60 cells were treated with the triterpenoids for 3 days. Total cell numbers were counted. The cell growth inhibition in the treated cells was compared with control cells. The concentration that inhibits 50% of growth was calculated. The data shown are means ± SE of three independent experiments.

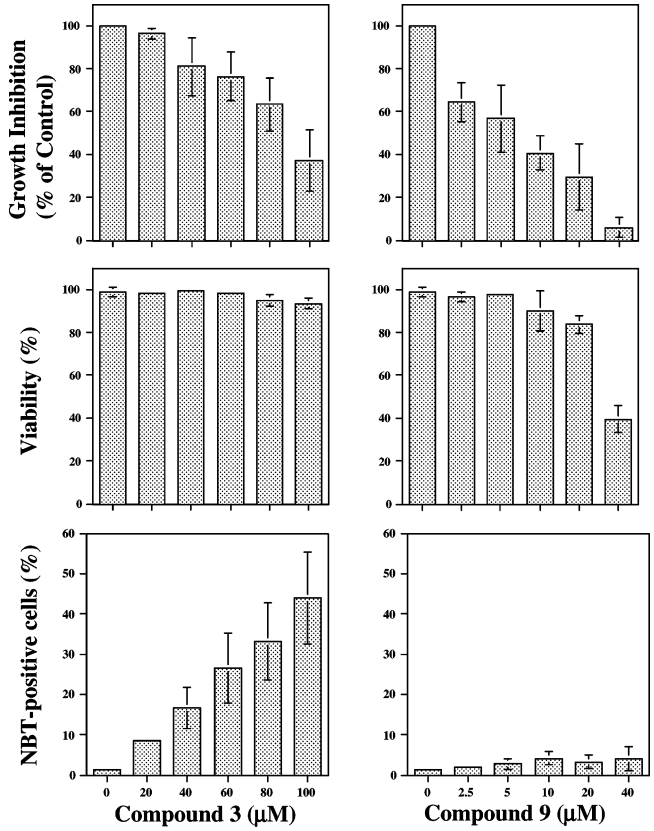

Figure 3. Dose-dependent growth inhibitory, cytotoxic, and differentiation effects of compounds **3** and **9** on HL-60 cells. Cells were treated with the indicated concentrations of each compound for 3 days. Cell growth inhibition of each treatment was compared with control cells. Percentages of trypan blue-negative (viable) cells and NBT-positive cells were calculated after counting 200 cells. Data shown are mean ± SE of three independent experiments.

treatment at different concentrations. As shown in Figure 3, compound **3** was less growth inhibitory, but it induced 44% NBT-positive cells after treatment at a concentration of 100 μM. In contrast, compound **9** was a potent growth inhibitory and cytotoxic agent without NBT reduction ability in HL-60 cells (Figure 3). The structure−activity relationship based on cell growth inhibition (Figure 1 and Table 3) and previous reports[8] suggests that oleanoic acid is the most effective cell growth inhibitor among these triterpenoids and that introduction of an additional hydroxyl group, an oxo, or converting a hydroxyl group into an oxo group decreases its cell growth inhibitory effect. Based on the NBT reduction assay,

a structure−activity relationship of differentiation activity among these triterpenoids could not be obtained. The differentiation effect of compound **3** without cytotoxicity suggests that it may be a novel differentiation inducer. Since about 50% of HL-60 cells become differentiated at an IG_{50} concentration, the growth arrestment of HL-60 by compound **3** may be due to differentiation induction. Since this compound does not show cytotoxicity, long-term treatment should increase its differentiation effect, which is worthy of further study.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were measured on a Yanaco MP-S3 micro-melting point apparatus. Optical rotations were measured with a Perkin-Elmer 241MC polarimeter. NMR spectra were recorded on a Bruker ARX 300 NMR spectrometer and a Bruker ARX 600 NMR spectrometer. The chemical shifts were quoted relative to TMS, and the coupling constants were in Hz. DEPT, HMBC, HSQC, COSY, and NOESY were measured on a Bruker ARX 600 NMR spectrometer. EIMS (70 eV) was conducted on a Shimadzu GCMS-QP5050A spectrometer. ESIMS was conducted on an Agilent 1100 SL instrument. HREIMS and HRFABMS were recorded on an Autospec-UltimaETOF instrument. IR was conducted on a Perkin IFS-55 spectrometer. The chromatographic silica gel (200−300 mesh) was produced by Qindao Ocean Chemical Factory, and Sephadex LH-20 was bought from GE Healthcare.

Plant Material. The resin of *S. tonkinensis* (Pier.) Craib was bought from Liaoning Medicinal Material Corporation, Shenyang, China, and identified by Prof. Qishi Sun of Shenyang Pharmaceutical University. A voucher specimen (ST 1230) was deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, China.

Extraction and Isolation. The resin of *S. tonkinensis* (900 g) was extracted with 95% EtOH. After removing solvent, a portion (150 g) of the residue was chromatographed on a column of silica gel with gradient elution using petroleum with increasing proportions of EtOAc and sequential solvent gradient from EtOAc to MeOH to give 10 fractions, fractions 1−10. Fraction 5 [petroleum−EtOAc (100:15)] was subsequently chromatographed over a silica gel column and Sephadex LH-20 [$CHCl_3$−MeOH (1:1)] to furnish compound **1** (15 mg). Repeated chromatography of fraction 6 [petroleum−EtOAc (100:20−50)] on silica gel columns afforded compounds **2** (70 mg), **3** (3 mg), and **4** (5 mg).

6*β*-Hydroxy-3-oxo-11α,12α-epoxyolean-28,13*β*-olide (1): colorless needles (acetone); mp > 300 °C; $[\alpha]^{20}_D$ +24.0 (*c* 0.75, $CHCl_3$); IR (KBr) ν_{max} 3568 (OH), 2932, 1755 (γ-lactone), 1708 (CO), 930, 872 cm^{-1} (epoxy); ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* (rel int) 484 $[M]^+$ (3.9), 249 (18), 204 (24), 189 (35); HREIMS *m*/*z* 484.3193 $[M]^+$ (calcd for $C_{30}H_{44}O_5$, 484.3189).

3*β*,6*β*-Dihydroxy-11α,12α-epoxyolean-28,13*β*-olide (2): amorphous white powder; $[\alpha]^{20}_D$ +42.0 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3561 (OH), 2933, 1752 (γ-lactone), 1148, 935, 872 (epoxy); ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* (rel int) 486 $[M]^+$ (3.94), 251 (17), 217 (14), 204 (59), 189 (31); HREIMS *m*/*z* 486.3337 $[M]^+$ (calcd for $C_{30}H_{46}O_5$ 486.3345).

3*β*,6*β*-Dihydroxy-11-oxo-olean-12-en-28-oic acid (3): white amorphous powder; $[\alpha]^{20}_D$ +38.4 (*c* 0.13, CH_3OH); IR (KBr) ν_{max} cm^{-1} 3446 (OH), 1700 (COOH), 1651 (CO); ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 509 $[M + Na]^+$, 321.3, 274.3; HRFABMS *m*/*z* 487.3423 $[M + H]^+$ (calcd for $C_{30}H_{46}O_5$ 486.3345).

3*β*-Hydroxy-12-oxo-13Hα-olean-28,19*β*-olide (4): colorless needles (acetone); mp 289−291°C; $[\alpha]^{20}_D$ +16.0 (*c* 0.25, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3472 (OH), 2932, 1784 (γ-lactone), 1685 (CO); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* (rel int) 470 $[M]^+$ (15), 249 (14), 207 (100), 204 (20), 189 (35), 175 (58), 107 (50); HREIMS *m*/*z* 470.3391 $[M]^+$ (calcd for $C_{30}H_{46}O_4$ 470.3396).

X-ray Crystallographic Analysis of Compound 4. A crystal of **4** with an appropriate size of 0.08 × 0.10 × 0.40 mm was selected for the X-ray investigation. Single-crystal data up to $\theta = 50°$ were collected on a MAC DIP-2030K diffractometer equipped with Mo Kα radiation. The cell constants were $a = 6.649$ Å, $b = 14.222$ Å, $c = 27.622$ Å, and space group as determined by systematic absences was monoclinic $P2_12_12_1$. A total of 2939 independent reflections were measured, out of which 2309 were recorded as observed ($|F|^2 > 3\sigma|F|^2$). An E map revealed all 34 non-hydrogen atoms. Refinement to convergence was carried out using a full matrix least squares approach and Fourier methods with a final *R* factor of 6.9%. A stereoscopic view of compound **4** is shown in Figure 2.

Cell Growth and Differentiation Assay. HL-60 cells were cultured in RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM L-glutamine, and 10% heat-inactivated fetal bovine serum. Cells in logarithmic growth were seeded at 1×10^5 cells/mL and were treated with different samples for 3 days. Studies were performed in triplicate. Cell viability was determined after staining with trypan blue. Trypan blue-stained (nonviable) cells and total cell number were determined with the aid of a hematocytometer. The growth inhibition in cells after treatment with different concentrations was calculated comparing with control cells, and a half growth inhibitory concentration (IG_{50}) was obtained by regression analysis of the concentration response data. The nitroblue tetrazolium (NBT) reduction assay as a determination of cell differentiation was performed as reported before.[13]

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Supporting Information Available: The X-ray crystallographic data for compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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