Hydroxylated Gedunin Derivatives from *Cedrela sinensis*

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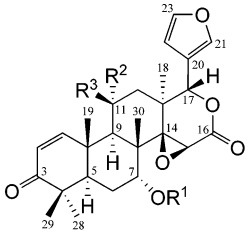
Four new limonoids, 11α-hydroxygedunin (**1**), 11*β*-hydroxygedunin (**2**), 7-deacetoxy-7α,11α-dihydroxygedunin (**3**), and 7-deacetoxy-7α,11*β*-dihydroxygedunin (**4**), were isolated from the cortex of *Cedrela sinensis*, together with three known compounds, gedunin (**5**), 7-deacetoxy-7α-hydroxygedunin (**6**), and 11-oxogedunin (**7**). The structures of **1**−**4** were determined by a combination of 2D NMR experiments and chemical methods and by X-ray crystallography of **1** and **2**.

Cedrela sinensis Juss. (Meliaceae) has been used in mainland China and Korea as a traditional medicine for the treatment of enteritis, dysentery, and itching. Previously, we reported isolation of five obacunone-type limonoids[1] and 23 apotirucallane-type triterpenoids[2] from the seeds, leaves, and stems of this plant. In the present study, we have isolated four new limonoids (**1**−**4**) along with three known limonoids, from the cortex of *C. sinensis* and have determined the structures of the new limonoids to be 11α-hydroxygedunin (**1**), 11*β*-hydroxygedunin (**2**), 7-deacetoxy-7α,11α-dihydroxygedunin (**3**), and 7-deacetoxy-7α,11*β*-dihydroxygedunin (**4**). The three known limonoids were shown to be gedunin (**5**), 7-deacetoxy-7α-hydroxygedunin (**6**), and 11-oxogedunin (**7**).



11α-Hydroxygedunin (**1**)
(R^1 = Ac, R^2 = OH, R^3 = H)
11β-Hydroxygedunin (**2**)
(R^1 = Ac, R^2 = H, R^3 = OH)
7-Deacetoxy-7α,11α-dihydroxygedunin (**3**)
(R^1 = H, R^2 = OH, R^3 = H)
7-Deacetoxy-7α,11β-dihydroxygedunin (**4**)
(R^1 = H, R^2 = H, R^3 = OH)
Gedunin (**5**)
(R^1 = Ac, R^2 = R^3 = H)
7-Deacetoxy-7α-hydroxygedunin (**6**)
(R^1 = H, R^2 = R^3 = H)
11-Oxogedunin (**7**)
(R^1 = Ac, R^2 = R^3 = O)
11α-Acetoxygedunin (**1a**)
(R^1 = Ac, R^2 = OAc, R^3 = H)
11β-Acetoxygedunin (**2a**)
(R^1 = Ac, R^2 = H, R^3 = OAc)

Results and Discussion

By a series of separation steps including Diaion HP-20, activated charcoal, and ODS column chromatography, and subsequent purification by preparative HPLC, a MeOH extract of the cortex of *C. sinensis* afforded four new limonoids, **1**−**4**, along with three known compounds, **5**−**7**.

Limonoid **1** was isolated as colorless prisms. Its molecular formula was determined to be $C_{28}H_{34}O_8$ from the $[M + H]^+$ peak at *m*/*z* 499.2293 (calcd for $C_{28}H_{35}O_8$, 499.2332) in the HRESIMS. Its NMR spectra generally resembled those of gedunin (**5**), suggesting that **1** has a gedunin-type limonoid skeleton.[3] The ^{1}H NMR spectrum of **1** showed the presence of five tertiary methyl groups (*δ* 1.06, 1.08, 1.17, 1.35, and 1.38), one acetate methyl group (*δ* 2.12, s), four oxymethine protons (*δ* 3.57, 4.45, 4.56, and 5.59), and a *β*-substituted furan ring (*δ* 6.36, 7.42, and 7.42, 1H each) (Table 1). The ^{13}C NMR spectrum indicated the presence of six methyls, two methylenes, 11 methines, and nine quaternary carbons, of which two at *δ* 167.4 and 169.9 were assigned to ester carbonyl carbons (Table 2). In the HMBC spectrum, the cross-peak between δ_C 169.9 and δ_H 4.45 (H-7) demonstrated that the acetoxy group is attached to C-7 (Figure 1). The ^{13}C NMR signals of C-1 (*δ* 161.4), C-2 (*δ* 123.9), and C-3 (*δ* 204.1) and the ^{1}H NMR signal of a pair of AB doublets at *δ* 5.79 and 8.18 (J = 10.5 Hz) suggested that the A-ring of **1** possesses a 1-en-3-one system. The cross-peaks between H-15/C-14, H-15/C-16, H-17/C-13, and H-17/C-14 in the HMBC spectrum suggested the presence of a *δ*-lactone group with a 14,15-epoxide in the D-ring. The C-11 signal of **1** appeared at *δ* 65.7 (d), implying that it is an oxymethine carbon. TPAP oxidation[4] of **1** afforded 11-oxogedunin (**7**), whereas acetylation of **1** afforded diacetate **1a**, which confirmed the presence of a hydroxyl group at C-11 in **1**. As regards the relative stereochemistry of **1**, NOE correlations detected between H-5/H-9, H-5/H_3-28, H-7/H_3-30, H-9/H_3-18, H-11/H_3-19, H-11/H_3-30, H-15/H_3-18, H_3-18/H-21, H_3-18/H-22, H_3-19/H_3-29, and H_3-19/H_3-30 showed that H-5, OAc-7, H-9, OH-11, Me-18, and Me-28 are α-oriented, whereas the 14,15-epoxide, H-17, Me-19, Me-29, and Me-30 groups are *β*-oriented (Figure 2). From these observations, limonoid **1** was determined to be 11α-hydroxygedunin. This structure was confirmed by X-ray crystallographic analysis (Figure 3).

Limonoid **2** was obtained as colorless prisms. Its molecular formula, $C_{28}H_{34}O_8$, as determined from the $[M + H]^+$ peak at *m*/*z* 499.2312 (calcd for $C_{28}H_{35}O_8$, 499.2332) in the HRESIMS, was the same as that of **1**, and the COSY and HMBC spectra were quite similar to those of **1**. The TPAP oxidation product of **2** was 11-oxogedunin (**7**), demonstrating that **1** and **2** have the same gross structure. The NOE correlation detected between H-11 and H_3-18 and the coupling constant of 4.6 Hz between H-9 and H-11 implied that the hydroxyl group at C-11 in **2** is *β*-oriented (Figure 4). Acetylation of **2** afforded a product, **2a**, for which the spectroscopic data were identical to those of the known compound 11*β*-acetoxygedunin.[5] Thus, **2** was determined to be 11*β*-hydroxygedunin. This structure was also confirmed by X-ray crystallographic analysis (Figure 5).

Limonoid **3** was obtained as an amorphous solid. From the $[M + H]^+$ peak at *m*/*z* 457.2224 (calcd for $C_{26}H_{33}O_7$, 457.2226) in the HRESIMS, its molecular formula was determined to be $C_{26}H_{32}O_7$. The ^{1}H and ^{13}C NMR spectra of **3** showed a close resemblance to those of **1**, implying that both compounds are of the same basic structure. The differences noted between the NMR spectra of these

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Table 1. ^{1}H NMR (500 MHz) Spectroscopic Data for Limonoids **1**−**4** in $CDCl_3$[a]

position	1	2	3	4
1	8.18 (d, 10.5)	7.39 (d, 10.2)	8.18 (d, 10.5)	7.38 (d, 10.3)
2	5.79 (d, 10.5)	5.91 (d, 10.2)	5.78 (d, 10.5)	5.90 (d, 10.3)
5	2.25 (m)	2.12 (m)	2.55 (dd, 13.4, 2.4)	2.42 (dd, 13.2, 2.1)
6α	1.95 (dt, 15.0, 2.9)	1.94 (m)	1.67 (dt, 14.6, 3.0)	1.67 (d-like, 13.7)
6*β*	1.76 (td, 15.0, 1.4)	1.94 (m)	1.89 (td, 14.6, 1.5)	2.05 (td, 13.7, 1.7)
7	4.45 (d, 1.4)	4.54 (t-like, 2.3)	3.49 (br s)	3.54 (d, 1.7)
9	2.62 (d, 9.6)	2.35 (d, 4.6)	2.63 (d, 9.7)	2.39 (d, 4.7)
11	4.56 (m)	4.82 (m)	4.54 (m)	4.80 (m)
12α	1.51 (d, 14.6)	2.13 (m)	1.47 (d, 14.6)	2.13 (dd, 13.4, 9.3)
12*β*	2.25 (m)	1.70 (m)	2.23 (dd, 14.6, 6.9)	1.68 (m)
15	3.57 (s)	3.57 (s)	3.99 (s)	3.84 (s)
17	5.59 (s)	5.66 (s)	5.57 (s)	5.65 (s)
18	1.38 (s)	1.19 (s)	1.36 (s)	1.19 (s)
19	1.35 (s)	1.63 (s)	1.32 (s)	1.60 (s)
21	7.42 (s)	7.42 (s)	7.42 (s)	7.41 (s)
22	6.36 (s)	6.36 (s)	6.36 (s)	6.36 (s)
23	7.42 (s)	7.42 (s)	7.42 (s)	7.42 (s)
28	1.06 (s)	1.07 (s)	1.15 (s)	1.15 (s)
29	1.08 (s)	1.07 (s)	1.11 (s)	1.10 (s)
30	1.17 (s)	1.38 (s)	1.10 (s)	1.31 (s)
OAc-7	2.12 (s)	2.07 (s)		

[a] Chemical shifts are reported in ppm relative to residual $CHCl_3$ resonance at 7.26 ppm. Multiplicity and *J* values in Hz are given in parentheses.

Table 2. ^{13}C NMR (125 MHz) Spectroscopic Data for Limonoids **1**−**4** in $CDCl_3$[a]

position	1	2	3	4
1	161.4	156.7	161.9	157.2
2	123.9	125.9	123.9	125.8
3	204.1	203.8	204.5	204.2
4	44.2	43.9	44.4	44.1
5	45.3	47.1	44.0	45.7
6	22.8	23.8	26.9	28.0
7	72.9	74.5	69.2	71.5
8	42.4	42.5	43.5	43.5
9	46.7	44.2	45.5	42.7
10	41.0	41.3	41.2	41.4
11	65.7	64.9	66.2	65.3
12	39.8	39.5	40.1	40.2
13	38.6	38.2	38.3	37.7
14	70.1	69.3	70.2	69.5
15	57.7	56.0	59.1	56.6
16	167.4	167.3	168.1	167.9
17	77.7	78.0	77.8	78.3
18	17.0	16.6	17.3	16.8
19	21.0	22.6	21.1	22.8
20	120.2	120.2	120.5	120.5
21	141.2	141.3	141.2	141.3
22	109.9	109.9	110.0	110.1
23	143.3	143.2	143.2	143.1
28	27.2	26.9	27.5	27.1
29	21.3	21.4	21.6	21.6
30	19.9	20.5	20.3	20.8
OAc-7 (C═O)	169.9	169.7		
OAc-7 (Me)	21.0	21.1		

[a] Chemical shifts are reported in ppm relative to the solvent resonance at 77.03 ppm.

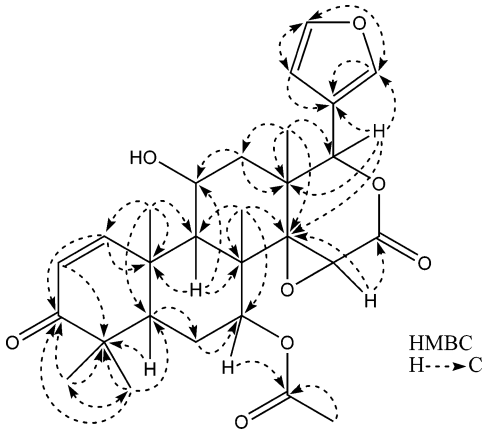

Figure 1. Selected HMBC correlations for **1**.

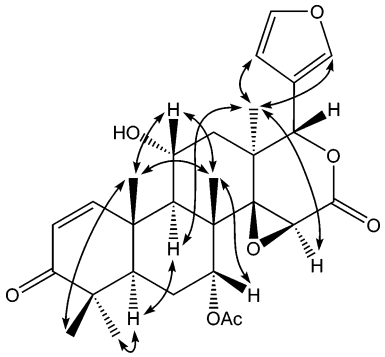

Figure 2. Selected NOE correlations for **1**.

limonoids were that **3** had no signal due to an acetyl group and that the C-7 signal of **3** (δ 69.2) was at a higher field than the corresponding C-7 signal of **1** (δ 72.9), demonstrating that **3** has no acetyl group at C-7. Deacetylation of **1** afforded a product that was shown to be identical to natural **3** from its spectoscopic data. From these facts, **3** was determined as 7-deacetoxy-7α,11α-dihydroxygedunin.

Limonoid **4** was obtained as an amorphous solid. Its molecular formula was determined to be $C_{26}H_{32}O_7$ from the $[M + H]^+$ peak at *m*/*z* 457.2194 (calcd for $C_{26}H_{33}O_7$, 457.2226) in the HRESIMS. The NMR spectra of **4** were generally similar to those of **3**, suggesting that they have the same basic structure. The spectroscopic differences between **3** and **4** were analogous to those observed between **1** and **2** (Tables 1 and 2). Deacetylation of **2** afforded a product whose spectroscopic data were shown to be identical to those of natural **4**. Thus, the structure of **4** was determined to be 7-deacetoxy-7α,11*β*-dihydroxygedunin.

Compounds **5**−**7** were identified as gedunin,[3,6] 7-deacetoxy-7α-hydroxygedunin,[7] and 11-oxogedunin,[8] respectively, by analysis of their spectroscopic data. Full ^{1}H and ^{13}C NMR data for the known compounds **6** and **7** are provided in the Experimental Section.

Compounds **2**, **2a**, and **4**−**7** all showed moderate cytotoxicity against P-388 murine leukemia cells, with IC_{50} values of 5.4, 3.8, 7.8, 3.3, 4.5, and 3.0 μg/mL, respectively, whereas compounds **1**, **1a**, and **3** had very weak or no activity, with IC_{50} values of 71, 63, and >100 μg/mL, respectively, suggesting that an oxygenated functionality at the 11α-position has a detrimental effect on the resultant cytotoxic activity.

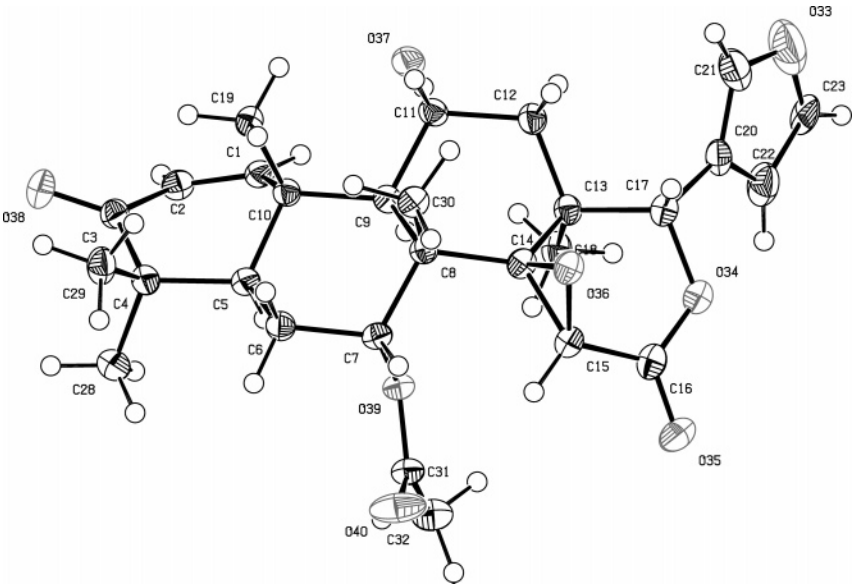

Figure 3. Crystal structure of **1**.

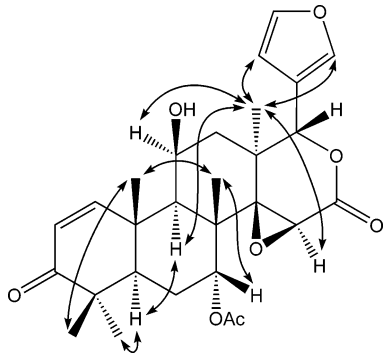

Figure 4. Selected NOE correlations for **2**.

Gedunin (**5**) is reported to have in vitro antimalarial activity against chloroquine-resistant K1 strain of *Plasmodium falciparum*[9] and thus has been regarded as a possible lead for new antimalarial drugs. Compounds **1**−**4**, having the same basic structure with a hydroxyl group at C-11, may be useful as a starting material for the preparation of new analogues of gedunin-related antimalarial drugs.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 digital polarimeter. UV spectra were taken on a JASCO V-530 spectrophotometer, IR spectra on a JASCO FT/IR 620 spectrophotometer, NMR spectra on a Bruker DRX-500 spectrometer at 300 K, and mass spectra on a Micromass LCT spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD system equipped with a SPD-10A UV detector (at 205 nm) and a reversed-phase column, Wakosil-II 5C18HG prep (5 μm, 20 × 250 mm), using mixed solvent systems of MeOH−H_2O or MeCN−H_2O, at a flow late of 10 mL/min. Single-crystal X-ray analysis was carried out on a Mac Science DIP diffractometer with Mo Kα radiation (λ = 0.71073 Å).

Plant Material. The cortex of *C. sinensis* was collected in Jilin Province, People's Republic of China, in September 2000, and the botanical origin was identified by Professor Soo-Cheol Kim of the Agricultural College of Yanbian University. A voucher specimen (00CHI005) has been deposited in the herbarium of Tokyo University of Pharmacy and Life Science.

Extraction and Isolation. The cut and air-dried cortex of *C. sinensis* (24 kg) was extracted with hot MeOH (3 × 90 L). After the removal of the solvent, the MeOH extract (3.2 kg) was placed on a column of HP-20 (8.0 kg) and fractionated into five fractions, by eluting with H_2O, 50% MeOH, 80% MeOH, MeOH, and acetone (each 90 L). The 80% MeOH fraction (198 g) was subjected to activated charcoal (450 g) column chromatography, eluting with MeOH, $CHCl_3$−MeOH (1:9), and $CHCl_3$−MeOH (1:1), with 30 L of each solvent used. The $CHCl_3$−MeOH (1:9) fraction (10 g) was further subjected to RP-18 (100 g) column chromatography, eluting with MeOH−H_2O (60:40, 80:20, 100:0, each 1 L), to afford three fractions (1−3). After removal of the solvent to dryness, fraction 1 (3.0 g) was subjected to HPLC using MeOH−H_2O (55:45) to afford two limonoid-containing fractions, each of which was subsequently purified by HPLC using MeCN−H_2O (35:65) to give **3** (1.5 mg) and **4** (1.2 mg), respectively. Fraction 2 (4.0 g) was subjected to HPLC using MeOH−H_2O (60:40) to give four limonoid-containing fractions. When subsequently purified by HPLC using MeCN−H_2O (40:60), the first limonoid-containing fraction gave **1** (191.8 mg) and **2** (42.7 mg), the second limonoid-containing fraction afforded 11-oxogedunin (**7**) (28.2 mg), and the third fraction yielded 7-deacetoxy-7α-hydroxygedunin (**6**) (66.0 mg). Fraction 3 (3.0 g) was subjected to HPLC using MeOH−H_2O (75:35) to give a limonoid-containing fraction, which was then purified by HPLC using MeCN−H_2O (43:57) to give gedunin (**5**) (286.7 mg).

11α-Hydroxygedunin (1): colorless prisms ($CHCl_3$−MeOH); mp 240−243 °C; $[\alpha]^{25}_D$ +18.5 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.20) nm; IR (film) ν_{max} 3650, 2965, 1795, 1701, 1679, 1651, 1539 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 499.2293 ($[M + H]^+$, calcd for $C_{28}H_{35}O_8$, 499.2332).

11β-Hydroxygedunin (2): colorless prisms ($CHCl_3$−MeOH); mp 294−297 °C; $[\alpha]^{25}_D$ +7.8 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ) 216 (4.20); IR (film) ν_{max} 3650, 2950, 1795, 1700, 1650, 1539 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 499.2312 ($[M + H]^+$, calcd for $C_{28}H_{35}O_8$, 499.2332).

7-Deacetoxy-7α,11α-dihydroxygedunin (3): amorphous solid; $[\alpha]^{25}_D$ +54.5 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.14) nm; IR (film) ν_{max} 3650, 2946, 1795, 1737, 1650, 1539 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 457.2224 ($[M + H]^+$, calcd for $C_{26}H_{33}O_7$, 457.2226).

7-Deacetoxy-7α,11β-dihydroxygedunin (4): amorphous solid; $[\alpha]^{25}_D$ +47.0 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.12) nm; IR (film) ν_{max} 3650, 2931, 1795, 1737, 1650, 1539 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 457.2194 ($[M + H]^+$, calcd for $C_{26}H_{33}O_7$, 457.2226).

Gedunin (5): colorless crystalline powder ($CHCl_3$−MeOH); mp 194−198 °C; $[\alpha]^{27}_D$ +42.8 (*c* 0.1, $CHCl_3$); HRESIMS *m*/*z* 483.2386 ($[M + H]^+$, calcd for $C_{28}H_{35}O_7$, 483.2383).

7-Deacetoxy-7α-hydroxygedunin (6): colorless crystalline powder ($CHCl_3$−MeOH); mp 270−275 °C; $[\alpha]^{28}_D$ +60.7 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.41 (1H, s, H-21), 7.40 (1H, d, J = 1.3 Hz, H-23), 7.10 (1H, d, J = 10.2 Hz, H-1), 6.35 (1H, s, H-22), 5.84 (1H, d, J = 10.2 Hz, H-2), 5.60 (1H, s, H-17), 3.91 (1H, s, H-15), 3.57 (1H, br s, H-7), 2.53 (1H, m, H-9), 2.48 (1H, m, H-5), 1.97 (1H, m, H-11α), 1.96 (1H, m, H-6β), 1.80 (1H, m, H-11β), 1.72 (1H, m,

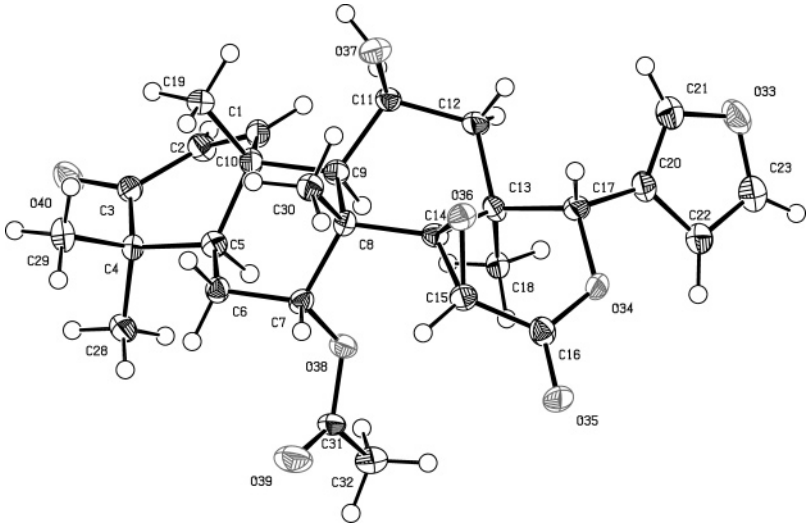

Figure 5. Crystal structure of **2**.

H-12β), 1.63 (1H, m, H-6α), 1.55 (1H, m, H-12α), 1.23 (3H, s, H_3-18), 1.20 (3H, s, H_3-19), 1.14 (3H, s, H_3-28), 1.09 (6H, s, H_3-29 and H_3-30); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.6 (C, C-3), 168.2 (C, C-16), 157.7 (CH, C-1), 143.0 (CH, C-23), 141.2 (CH, C-21), 125.8 (CH, C-2), 120.7 (C, C-20), 110.0 (CH, C-22), 78.5 (CH, C-17), 70.0 (C, C-14), 69.8 (CH, C-7), 57.9 (CH, C-15), 44.6 (CH, C-5), 44.2 (C, C-4), 43.7 (C, C-8), 40.2 (C, C-10), 38.4 (C, C-13), 38.0 (CH, C-9), 27.3 (CH_3, C-28), 27.3 (CH_2, C-6), 26.4 (CH_2, C-12), 21.5 (CH_3, C-29), 19.9 (CH_3, C-19), 18.7 (CH_3, C-30), 17.8 (CH_3, C-18), 15.1 (CH_2, C-11); HRESIMS *m*/*z* 441.2316 ([M + H]$^+$, calcd for $C_{26}H_{33}O_6$, 441.2277).

11-Oxogedunin (7): amorphous solid; $[\alpha]^{28}_D$ −38.1 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.45 (1H, s, H-21), 7.44 (1H, s, H-23), 7.31 (1H, d, J = 10.2 Hz, H-1), 6.35 (1H, s, H-22), 5.85 (1H, d, J = 10.2 Hz, H-2), 5.68 (1H, s, H-17), 4.68 (1H, t-like, J = 2.5 Hz, H-7), 3.66 (1H, s, H-15), 3.35 (1H, s, H-9), 2.47 (1H, d, J = 19.0 Hz, H-12α), 2.30 (1H, d, J = 19.0 Hz, H-12β), 2.14 (3H, s, OAc-7), 2.07 (1H, dd, J = 13.3, 2.0 Hz, H-5), 1.97 (1H, m, H-6α), 1.84 (1H, ddd, J = 14.9, 13.3, 1.9 Hz, H-6β), 1.47 (3H, s, H_3-19), 1.45 (3H, s, H_3-18), 1.17 (3H, s, H_3-30), 1.08 (3H, s, H_3-28), 1.07 (3H, s, H_3-29); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 205.5 (C, C-11), 203.2 (C, C-3), 169.4 (C, O*C*OCH$_3$-7), 166.3 (C, C-16), 158.0 (CH, C-1), 143.7 (CH, C-23), 141.3 (CH, C-21), 125.9 (CH, C-2), 119.4 (C, C-20), 109.4 (CH, C-22), 77.1 (CH, C-17), 72.8 (CH, C-7), 68.5 (C, C-14), 56.0 (CH, C-9), 55.6 (CH, C-15), 45.8 (CH_2, C-12), 45.5 (CH, C-5), 44.1 (C, C-4), 43.6 (C, C-8), 38.9 (C, 2C, C-10 and C-13), 27.1 (CH_3, C-28), 23.4 (CH_2, C-6), 21.2 (CH_3, C-29), 21.1 (CH_3, C-19), 21.0 (CH_3, OCO*C*H$_3$-7), 20.2 (CH_3, C-30), 18.5 (CH_3, C-18); HRESIMS *m*/*z* 497.2187 ([M + H]$^+$, calcd for $C_{28}H_{33}O_8$, 497.2175).

Oxidation of 1. Solid TPAP (tetrapropylammonium per-ruthenate, 0.6 mg, 0.0017 mmol) was added in one portion to a stirred mixture of **1** (9.0 mg, 0.018 mmol), 4-methylmorpholine *N*-oxide (3.2 mg, 0.027 mmol), and powdered 4 Å molecular sieves (9.0 mg) in CH_2Cl_2 (2 mL), and the whole was stirred at room temperature for 7 h. The mixture was diluted with $CHCl_3$ and washed sequentially with aqueous Na_2SO_3 and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was subjected to ODS-HPLC with MeCN−H_2O (43:57) to afford an oxidation product (8.5 mg, 95%), which was identified as 11-oxogedunin (**7**) by comparison of their NMR and mass spectra.

Oxidation of 2. TPAP oxidation of **2** (2.2 mg) by the procedure described above gave an oxidation product (2.0 mg, 91%), which was shown to be identical to 11-oxogedunin (**7**) by comparison of their NMR and mass spectra.

Acetylation of 1. Acetic anhydride (1 mL) was added to a solution of **1** (7.0 mg) in pyridine (1 mL), and the mixture was left at room temperature for 24 h. After removal of the volatiles in vacuo, the residue was subjected to ODS-HPLC with MeOH−H_2O (60:40) to afford **1a** (6.5 mg, 86%) as an amorphous solid: $[\alpha]^{25}_D$ +6.1 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (2H, s, H-21 and H-23), 7.36 (1H, d, J = 10.5 Hz, H-1), 6.32 (1H, s, H-22), 5.81 (1H, d, J = 10.5 Hz, H-2), 5.59 (1H, s, H-17), 5.55 (1H, t-like, J = 9.1 Hz, H-11), 4.49 (1H, br s, H-7), 3.59 (1H, s, H-15), 2.87 (1H, d, J = 10.6 Hz, H-9), 2.30 (1H, dd, J = 15.3, 7.5 Hz, H-12β), 2.24 (1H, m, H-5), 2.13 (3H, s, OAc-7), 2.06 (3H, s, OAc-11), 1.98 (1H, d-like, J = 14.6 Hz, H-6α), 1.78 (1H, t-like, J = 14.6 Hz, H-6β), 1.46 (1H, d, J = 15.3 Hz, H-12α), 1.33 (3H, s, H_3-18), 1.29 (3H, s, H_3-19), 1.22 (3H, s, H_3-30), 1.09 (3H, s, H_3-29), 1.06 (3H, s, H_3-28); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 203.4 (C, C-3), 169.8 (C, 2C, O*C*OCH$_3$-7 and -11), 167.0 (C, C-16), 158.3 (CH, C-1), 143.4 (CH, C-23), 141.3 (CH, C-21), 124.9 (CH, C-2), 120.0 (C, C-20), 109.6 (CH, C-22), 77.5 (CH, C-17), 72.7 (CH, C-7), 69.7 (C, C-14), 68.5 (CH, C-11), 57.6 (CH, C-15), 45.1 (CH, C-5), 44.4 (C, C-4), 43.9 (CH, C-9), 42.4 (C, C-8), 40.8 (C, C-10), 38.3 (C, C-13), 36.6 (CH_2, C-12), 27.3 (CH_3, C-28), 22.7 (CH_2, C-6), 21.7 (CH_3, OCO*C*H$_3$-11), 21.2 (CH_3, C-29), 21.0 (CH_3, OCO*C*H$_3$-7), 20.9 (CH_3, C-19), 19.9 (CH_3, C-30), 17.4 (CH_3, C-18); HRESIMS *m*/*z* 541.2410 ([M + H]$^+$, calcd for $C_{30}H_{37}O_9$, 541.2438).

Acetylation of 2. Acetylation of **2** (3.0 mg) by the procedure described above gave **2a** (2.8 mg, 86%) as an amorphous solid, which was identified as 11β-acetoxygedunin by comparing its ^{1}H NMR and mass spectra and optical rotations with those in the literature:[5] $[\alpha]^{25}_D$ +32.9 (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (1H, s, H-23), 7.40 (1H, s, H-21), 7.12 (1H, d, J = 10.3 Hz, H-1), 6.33 (1H, s, H-22), 5.91 (1H, d, J = 10.3 Hz, H-2), 5.80 (1H, m, H-11), 5.61 (1H, s, H-17), 4.59 (1H, br s, H-7), 3.59 (1H, s, H-15), 2.54 (1H, d, J = 4.8 Hz, H-9), 2.32 (1H, dd, J = 14.1, 9.8 Hz, H-12α), 2.14 (1H, m, H-5), 2.14 (3H, s, OAc-11), 2.08 (3H, s, OAc-7), 1.94 (2H, m, H-6α and H-6β), 1.54 (1H, m, H-12β), 1.45 (3H, s, H_3-19), 1.37 (3H, s, H_3-30), 1.24 (3H, s, H_3-18), 1.07 (3H, s, H_3-28), 1.06 (3H, s, H_3-29); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 203.2 (C, C-3), 169.9 (C, O*C*OCH$_3$-11), 169.6 (C, O*C*OCH$_3$-7), 166.9 (C, C-16), 155.0 (CH, C-1), 143.4 (CH, C-23), 141.3 (CH, C-21), 126.5 (CH, C-2), 119.9 (C, C-20), 109.8 (CH, C-22), 78.0 (CH, C-17), 74.3 (CH, C-7), 68.9 (C, C-14), 66.4 (CH, C-11), 55.7 (CH, C-15), 47.2 (CH, C-5), 43.9 (C, C-4), 43.4 (CH, C-9), 42.4 (C, C-8), 40.7 (C, C-10), 37.8 (C, C-13), 36.1 (CH_2, C-12), 27.0 (CH_3, C-28), 23.8 (CH_2, C-6), 21.8 (CH_3, C-19), 21.6 (CH_3, OCO*C*H$_3$-11), 21.4 (CH_3, C-29), 21.1 (CH_3, OCO*C*H$_3$-7), 20.2 (CH_3, C-30), 16.9 (CH_3, C-18); HRESIMS *m*/*z* 541.2421 ([M + H]$^+$, calcd for $C_{30}H_{37}O_9$, 541.2438).

Deacetylation of 1. Potassium carbonate (1.0 mg) was added to a solution of **1** (2.0 mg) in MeOH (1 mL), and the mixture was stirred at room temperature for 24 h. The mixture was diluted with $CHCl_3$, washed sequentially with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by ODS-HPLC using MeCN−H_2O (40:60) to give a product (1.6 mg, 87%) that was shown to be identical to natural **3** by comparison of their NMR and mass spectra.

Deacetylation of 2. Deacetylation of **2** (1.1 mg) by the procedure described above gave a product (0.9 mg, 89%) whose NMR and mass spectra showed that the product was identical to natural **4**.

X-ray Crystallographic Study of 1. $C_{28}H_{34}O_8$, M = 498.55, 0.50 × 0.30 × 0.30 mm, orthorhombic, $P2_12_12_1$, a = 11.7970(4) Å, b =

12.0160(4) Å, c = 34.2630(5) Å, V = 4856.9(2) Å,^{3} Z = 8, D_x = 1.364 Mg m^{-3}, μ(Mo Kα) = 0.099 mm^{-1}, 5911 measured reflections, 5911 unique reflections, 4698 observed reflections [$I > 2\sigma(I)$], R1 = 0.0434, wR2 = 0.1026 (observed data), GOF = 0.913; R1 = 0.0522, wR2 = 0.1053 (all data). The absolute structure could not be determined crystallographically.

X-ray Crystallographic Study of 2. $C_{28}H_{34}O_8$, M = 498.55, 0.50 × 0.44 × 0.20 mm, monoclinic, $P2_1$, a = 9.6670(11) Å, b = 12.593-(2) Å, c = 10.2860(16) Å, β = 103.447(9)°, V = 1217.9(3) Å,^{3} Z = 2, D_x = 1.360 Mg m^{-3}, μ(Mo Kα) = 0.099 mm^{-1}, 2791 measured reflections, 2791 unique reflections, 2019 observed reflections [$I > 2\sigma(I)$], R1 = 0.0411, wR2 = 0.0954 (observed data), GOF = 0.912; R1 = 0.0561, wR2 = 0.0995 (all data). The absolute structure could not be determined crystallographically.

The structures were solved by direct methods using the maXus crystallographic software package[10] and refined by full-matrix least-squares on F^2 using the program SHELXL-97.[11]

CCDC 604386 and 604387 contain the supplementary crystallographic data for compounds **1** and **2**, respectively, from this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Cytotoxicity Assays. Evaluation of cytotoxicity against P-388 murine leukemia cells was performed as described previously.[12]

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