

Hydroxylated Gedunin Derivatives from *Cedrela sinensis*

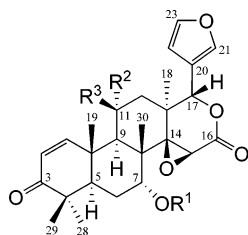
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Four new limonoids, 11 α -hydroxygedunin (**1**), 11 β -hydroxygedunin (**2**), 7-deacetoxy-7 α ,11 α -dihydroxygedunin (**3**), and 7-deacetoxy-7 α ,11 β -dihydroxygedunin (**4**), were isolated from the cortex of *Cedrela sinensis*, together with three known compounds, gedunin (**5**), 7-deacetoxy-7 α -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 limonoids¹ and 23 apotirucallane-type triterpenoids² 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 11 α -hydroxygedunin (**1**), 11 β -hydroxygedunin (**2**), 7-deacetoxy-7 α ,11 α -dihydroxygedunin (**3**), and 7-deacetoxy-7 α ,11 β -dihydroxygedunin (**4**). The three known limonoids were shown to be gedunin (**5**), 7-deacetoxy-7 α -hydroxygedunin (**6**), and 11-oxogedunin (**7**).



- 11 α -Hydroxygedunin (**1**)
(R¹ = Ac, R² = OH, R³ = H)
11 β -Hydroxygedunin (**2**)
(R¹ = Ac, R² = H, R³ = OH)
7-Deacetoxy-7 α ,11 α -dihydroxygedunin (**3**)
(R¹ = H, R² = OH, R³ = H)
7-Deacetoxy-7 α ,11 β -dihydroxygedunin (**4**)
(R¹ = H, R² = H, R³ = OH)
Gedunin (**5**)
(R¹ = Ac, R² = R³ = H)
7-Deacetoxy-7 α -hydroxygedunin (**6**)
(R¹ = H, R² = R³ = H)
11-Oxogedunin (**7**)
(R¹ = Ac, R² = R³ = O)
11 α -Acetoxygedunin (**1a**)
(R¹ = Ac, R² = OAc, R³ = H)
11 β -Acetoxygedunin (**2a**)
(R¹ = Ac, R² = H, R³ = 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 C₂₈H₃₄O₈ from the [M + H]⁺ peak

at *m/z* 499.2293 (calcd for C₂₈H₃₅O₈, 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 ¹H 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 ¹³C 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 ¹³C NMR signals of C-1 (δ 161.4), C-2 (δ 123.9), and C-3 (δ 204.1) and the ¹H 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 oxidation⁴ 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/H₃-28, H-7/H₃-30, H-9/H₃-18, H-11/H₃-19, H-11/H₃-30, H-15/H₃-18, H₃-18/H-21, H₃-18/H-22, H₃-19/H₃-29, and H₃-19/H₃-30 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 11 α -hydroxygedunin. This structure was confirmed by X-ray crystallographic analysis (Figure 3).

Limonoid **2** was obtained as colorless prisms. Its molecular formula, C₂₈H₃₄O₈, as determined from the [M + H]⁺ peak at *m/z* 499.2312 (calcd for C₂₈H₃₅O₈, 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 H₃-18 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 C₂₆H₃₃O₇, 457.2226) in the HRESIMS, its molecular formula was determined to be C₂₆H₃₂O₇. The ¹H and ¹³C 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. ^1H 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)
6 α	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)
12 α	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.

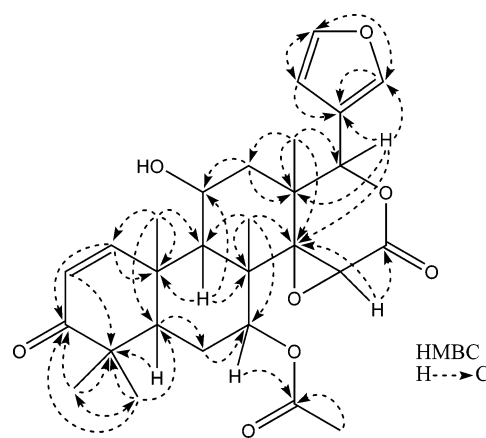
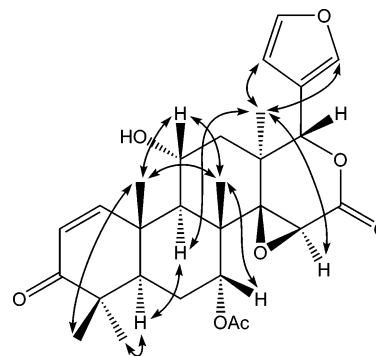
Table 2. ^{13}C NMR (125 MHz) Spectroscopic Data for Limonoids **1–4** in CDCl_3^a

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.

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 spectroscopic data. From these facts, **3** was determined as 7-deacetoxy-7 α ,11 α -dihydroxygedunin.

Limonoid **4** was obtained as an amorphous solid. Its molecular formula was determined to be $\text{C}_{26}\text{H}_{32}\text{O}_7$ from the $[\text{M} + \text{H}]^+$ peak at m/z 457.2194 (calcd for $\text{C}_{26}\text{H}_{33}\text{O}_7$, 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

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

identical to those of natural **4**. Thus, the structure of **4** was determined to be 7-deacetoxy-7 α ,11 β -dihydroxygedunin.

Compounds **5–7** were identified as gedunin,^{3,6} 7-deacetoxy-7 α -hydroxygedunin,⁷ and 11-oxogedunin,⁸ respectively, by analysis of their spectroscopic data. Full ^1H 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 $\mu\text{g/mL}$, respectively, whereas compounds **1**, **1a**, and **3** had very weak or no activity, with IC_{50} values of 71, 63, and >100 $\mu\text{g/mL}$, respectively, suggesting that an oxygenated functionality at the 11 α -position has a detrimental effect on the resultant cytotoxic activity.

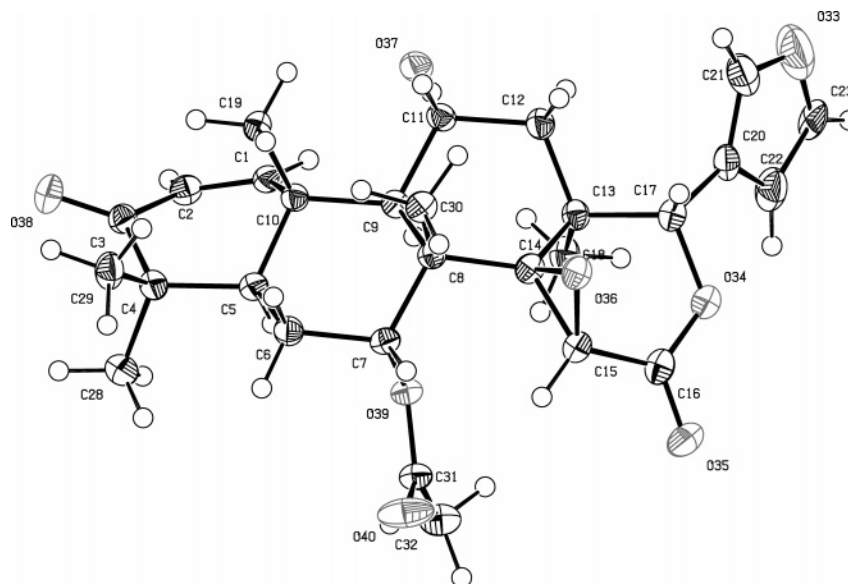


Figure 3. Crystal structure of **1**.

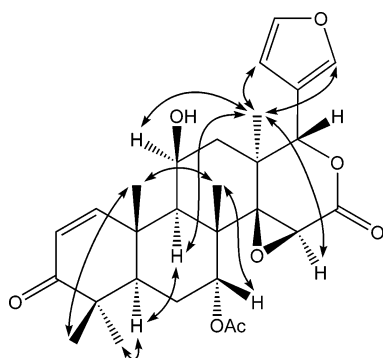


Figure 4. Selected NOE correlations for **2**.

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 μ m, 20 \times 250 mm), using mixed solvent systems of MeOH–H₂O or MeCN–H₂O, at a flow rate of 10 mL/min. Single-crystal X-ray analysis was carried out on a Mac Science DIP diffractometer with Mo K α 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 \times 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 H₂O, 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, CHCl₃–MeOH (1:9), and CHCl₃–MeOH (1:1), with 30 L of each solvent used. The CHCl₃–MeOH (1:9) fraction (10 g) was further subjected to RP-18 (100 g) column chromatography, eluting with MeOH–H₂O (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–H₂O (55:45) to afford two limonoid-containing fractions, each of which was subsequently purified by HPLC using MeCN–H₂O (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–H₂O (60:40) to give four limonoid-containing fractions. When subsequently purified by HPLC using MeCN–H₂O (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-7 α -hydroxygedunin (**6**) (66.0 mg). Fraction 3 (3.0 g) was subjected to HPLC using MeOH–H₂O (75:35) to give a limonoid-containing fraction, which was then purified by HPLC using MeCN–H₂O (43:57) to give gedunin (**5**) (286.7 mg).

11 α -Hydroxygedunin (1): colorless prisms (CHCl₃–MeOH); mp 240–243 °C; [α]_D²⁵ +18.5 (c 0.3, CHCl₃); 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 ¹³C NMR, see Tables 1 and 2; HRESIMS m/z 499.2293 ([M + H]⁺, calcd for C₂₈H₃₅O₈, 499.2332).

11 β -Hydroxygedunin (2): colorless prisms (CHCl₃–MeOH); mp 294–297 °C; [α]_D²⁵ +7.8 (c 0.1, CHCl₃); UV (MeOH) λ_{\max} nm (log ϵ) 216 (4.20); IR (film) ν_{\max} 3650, 2950, 1795, 1700, 1650, 1539 cm^{–1}; ¹H and ¹³C NMR, see Tables 1 and 2; HRESIMS m/z 499.2312 ([M + H]⁺, calcd for C₂₈H₃₅O₈, 499.2332).

7-Deacetoxy-7 α ,11 α -dihydroxygedunin (3): amorphous solid; [α]_D²⁵ +54.5 (c 0.06, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 215 (4.14) nm; IR (film) ν_{\max} 3650, 2946, 1795, 1737, 1650, 1539 cm^{–1}; ¹H and ¹³C NMR, see Tables 1 and 2; HRESIMS m/z 457.2224 ([M + H]⁺, calcd for C₂₆H₃₃O₇, 457.2226).

7-Deacetoxy-7 α ,11 β -dihydroxygedunin (4): amorphous solid; [α]_D²⁵ +47.0 (c 0.04, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 215 (4.12) nm; IR (film) ν_{\max} 3650, 2931, 1795, 1737, 1650, 1539 cm^{–1}; ¹H and ¹³C NMR, see Tables 1 and 2; HRESIMS m/z 457.2194 ([M + H]⁺, calcd for C₂₆H₃₃O₇, 457.2226).

Gedunin (5): colorless crystalline powder (CHCl₃–MeOH); mp 194–198 °C; [α]_D²⁷ +42.8 (c 0.1, CHCl₃); HRESIMS m/z 483.2386 ([M + H]⁺, calcd for C₂₈H₃₅O₇, 483.2383).

7-Deacetoxy-7 α -hydroxygedunin (6): colorless crystalline powder (CHCl₃–MeOH); mp 270–275 °C; [α]_D²⁸ +60.7 (c 0.1, CHCl₃); ¹H NMR (CDCl₃, 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-11 α), 1.96 (1H, m, H-6 β), 1.80 (1H, m, H-11 β), 1.72 (1H, m,

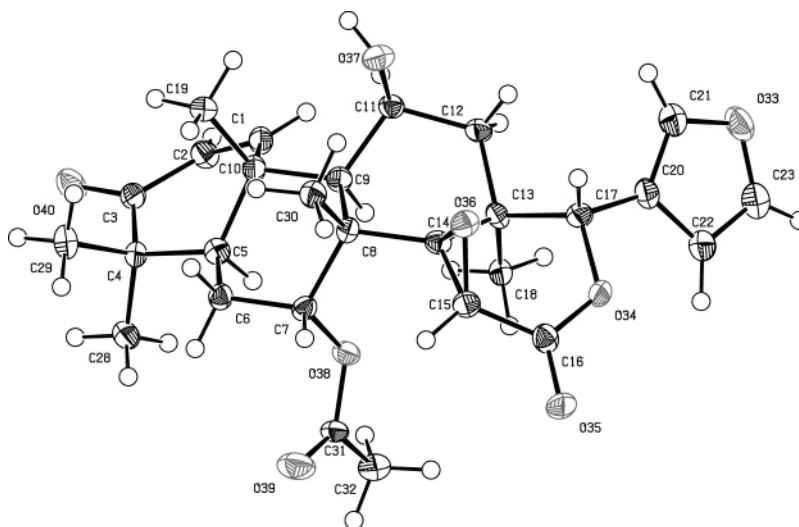


Figure 5. Crystal structure of **2**.

H-12 β), 1.63 (1H, m, H-6 α), 1.55 (1H, m, H-12 α), 1.23 (3H, s, H₃-18), 1.20 (3H, s, H₃-19), 1.14 (3H, s, H₃-28), 1.09 (6H, s, H₃-29 and H₃-30); ¹³C NMR (CDCl₃, 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 (CH₃, C-28), 27.3 (CH₂, C-6), 26.4 (CH₂, C-12), 21.5 (CH₃, C-29), 19.9 (CH₃, C-19), 18.7 (CH₃, C-30), 17.8 (CH₃, C-18), 15.1 (CH₂, C-11); HRESIMS m/z 441.2316 ([M + H]⁺, calcd for C₂₆H₃₃O₆, 441.2277).

11-Oxogedunin (7): amorphous solid; [α]_D²⁵ −38.1 (c 0.1, CHCl₃); ¹H NMR (CDCl₃, 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-12 α), 2.30 (1H, d, J = 19.0 Hz, H-12 β), 2.14 (3H, s, OAc-7), 2.07 (1H, dd, J = 13.3, 2.0 Hz, H-5), 1.97 (1H, m, H-6 α), 1.84 (1H, ddd, J = 14.9, 13.3, 1.9 Hz, H-6 β), 1.47 (3H, s, H₃-19), 1.45 (3H, s, H₃-18), 1.17 (3H, s, H₃-30), 1.08 (3H, s, H₃-28), 1.07 (3H, s, H₃-29); ¹³C NMR (CDCl₃, 125 MHz) δ 205.5 (C, C-11), 203.2 (C, C-3), 169.4 (C, OCOCH₃-7), 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 (CH₂, C-12), 45.5 (CH, C-5), 44.1 (C, C-4), 43.6 (C, C-8), 38.9 (C, C-10 and C-13), 27.1 (CH₃, C-28), 23.4 (CH₂, C-6), 21.2 (CH₃, C-29), 21.1 (CH₃, C-19), 21.0 (CH₃, OCOCH₃-7), 20.2 (CH₃, C-30), 18.5 (CH₃, C-18); HRESIMS m/z 497.2187 ([M + H]⁺, calcd for C₂₈H₃₃O₈, 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 CH₂Cl₂ (2 mL), and the whole was stirred at room temperature for 7 h. The mixture was diluted with CHCl₃ and washed sequentially with aqueous Na₂SO₃ and brine. The organic layer was dried (MgSO₄), filtered, and concentrated. The residue was subjected to ODS-HPLC with MeCN–H₂O (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–H₂O (60:40) to afford **1a** (6.5 mg, 86%) as an amorphous solid: [α]_D²⁵ +6.1 (c 0.3, CHCl₃); ¹H NMR (CDCl₃, 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-12 β), 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-6 α), 1.78 (1H, t-like, J = 14.6 Hz, H-6 β), 1.46 (1H, d, J = 15.3 Hz, H-12 α), 1.33 (3H, s, H₃-18), 1.29 (3H, s, H₃-19), 1.22 (3H, s, H₃-30), 1.09 (3H, s, H₃-29), 1.06 (3H, s, H₃-28); ¹³C NMR (CDCl₃, 125 MHz) δ 203.4 (C, C-3), 169.8 (C, 2C, OCOCH₃-7 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 (CH₂, C-12), 27.3 (CH₃, C-28), 22.7 (CH₂, C-6), 21.7 (CH₃, OCOCH₃-11), 21.2 (CH₃, C-29), 21.0 (CH₃, OCOCH₃-7), 20.9 (CH₃, C-19), 19.9 (CH₃, C-30), 17.4 (CH₃, C-18); HRESIMS m/z 541.2410 ([M + H]⁺, calcd for C₃₀H₃₇O₉, 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 11 β -acetoxygedunin by comparing its ¹H NMR and mass spectra and optical rotations with those in the literature:⁵ [α]_D²⁵ +32.9 (c 0.2, CHCl₃); ¹H NMR (CDCl₃, 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-12 α), 2.14 (1H, m, H-5), 2.14 (3H, s, OAc-11), 2.08 (3H, s, OAc-7), 1.94 (2H, m, H-6 α and H-6 β), 1.54 (1H, m, H-12 β), 1.45 (3H, s, H₃-19), 1.37 (3H, s, H₃-30), 1.24 (3H, s, H₃-18), 1.07 (3H, s, H₃-28), 1.06 (3H, s, H₃-29); ¹³C NMR (CDCl₃, 125 MHz) δ 203.2 (C, C-3), 169.9 (C, OCOCH₃-11), 169.6 (C, OCOCH₃-7), 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 (CH₂, C-12), 27.0 (CH₃, C-28), 23.8 (CH₂, C-6), 21.8 (CH₃, C-19), 21.6 (CH₃, OCOCH₃-11), 21.4 (CH₃, C-29), 21.1 (CH₃, OCOCH₃-7), 20.2 (CH₃, C-30), 16.9 (CH₃, C-18); HRESIMS m/z 541.2421 ([M + H]⁺, calcd for C₃₀H₃₇O₉, 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 CHCl₃, washed sequentially with H₂O and brine, dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The residue was purified by ODS-HPLC using MeCN–H₂O (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. C₂₈H₃₄O₈, M = 498.55, 0.50 \times 0.30 \times 0.30 mm, orthorhombic, $P2_12_12_1$, a = 11.7970(4) Å, b =

12.0160(4) Å, $c = 34.2630(5)$ Å, $V = 4856.9(2)$ Å³, $Z = 8$, $D_x = 1.364$ Mg m⁻³, $\mu(\text{Mo K}\alpha) = 0.099$ 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. C₂₈H₃₄O₈, $M = 498.55$, $0.50 \times 0.44 \times 0.20$ mm, monoclinic, $P2_1$, $a = 9.6670(11)$ Å, $b = 12.593(2)$ Å, $c = 10.2860(16)$ Å, $\beta = 103.447(9)^\circ$, $V = 1217.9(3)$ Å³, $Z = 2$, $D_x = 1.360$ Mg m⁻³, $\mu(\text{Mo K}\alpha) = 0.099$ 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 package¹⁰ 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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