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〔Article〕

Synthesis of analogues through redox reactions at position 16 of Gedunin and 7–Deacetoxy–7α –hydroxygedunin

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Abstract

Of the gedunin-type limonoids obtained from Chinese toon, or *Cedrela sinensis* Juss. (Meliaceae), gedunin (**1**)[1] is a substance that can be isolated in relatively large quantities. Acetylating gedunin (**1**) readily results in 7–deacetoxy–7α –hydroxygedunin (**2**). A previous study[2] obtained gedunin analogues by reducing the 1–en–3–one structure of the A ring of gedunin (**1**), but no studies have obtained analogues by reducing the carbonyl group at position 16 on the D ring of gedunin (**1**) or 7–deacetoxy–7α –hydroxygedunin (**2**). In order to determine the chemical reactivity of lactone on the D ring of gedunin (**1**) and 7–deacetoxy–7α –hydroxygedunin (**2**), the current authors facilitated redox reactions at position 16. This allowed the synthesis of 2 known compounds, 7–oxogedunin (**5**) and 3 β –hydroxygedunin (**9**), as well as 5 new analogues (compounds **3**, **4**, **6**, **7**, and **8**), as reported here. In order to determine the reactivity of gedunin (**1**), an attempt was made to reduce the substance with $LiBH_4$ and $NaBH_4$. $LiBH_4$ and $NaBH_4$ reduced the ketone at position 3 as well as the lactone-carbonyl group at position 16, yielding a hemiacetal. Further reduction failed to result in a 16–methylol form, indicating that this hemiacetal structure is highly stable. When compound **8** was oxidized with MnO_2, the hemiacetal hydroxyl group at position 16 was more readily oxidized than the allyl alcohol at position 3. Limonoid analogues not found in nature were synthesized in order to determine the cytotoxic activity of different functional groups against tumor cells, and the cytotoxic activity of these compounds against P–388 murine leukemia cells was examined. In compounds **3**–**8**, the carbonyl group at position 16 of the D ring of gedunin (**1**) was oxidized or reduced, and results indicated that these compounds lacked cytotoxic activity for the most part.

Keywords: Gedunin-type limonoid, 7–Deacetoxy–7α –hydroxygedunin, Redox reactions, P388 murine leukemia cells

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Gedunin および 7-deacetoxy-7 α -hydroxygedunin の 16 位酸化還元反応によるアナログ合成について

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1. Introduction

The mahogany family is known to contain large quantities of limonoids (a unique secondary metabolite with a 4,4,8-trimethyl-17-furanylsteroid skeleton) and triterpene compounds produced by precursors of limonoids. Limonoids are found in the mahogany family (Meliaceae), in some members of the rue family (Rutaceae) and the Mediterranean relict shrub family (Cneoraceae), and the East African shrub *Harrisonia abbysinica* (Simaroubaceae) . Currently, over 300 limonoids have been isolated, and these compounds are anticipated to have useful bioactivities such as deterring insect feeding, antibacterial activity, and antiviral activity. Gedunin-type compounds are limonoids from the mahogany family, and previous studies have reported that these compounds have antimalarial activity[3,4,5] as well as biological activity, e.g. inhibiting insect growth[2] or intake[6].

A member of the mahogany family, the Chinese toon, or *Cedrela sinensis* Juss., is a tall deciduous tree in the genus *Cedrela* native to China. The tree grows up to about 30 m tall, and its leaves are 30–50 cm long and pinnate, with an even or odd number of leaflets. Leaflets are 10–20 cm long and elliptical. The leaves are used as vegetables and as medicine. White flower clusters are produced in large panicles in June to July. The timber is hard and shiny, and it is used as a building material, to make furniture, and for carving.

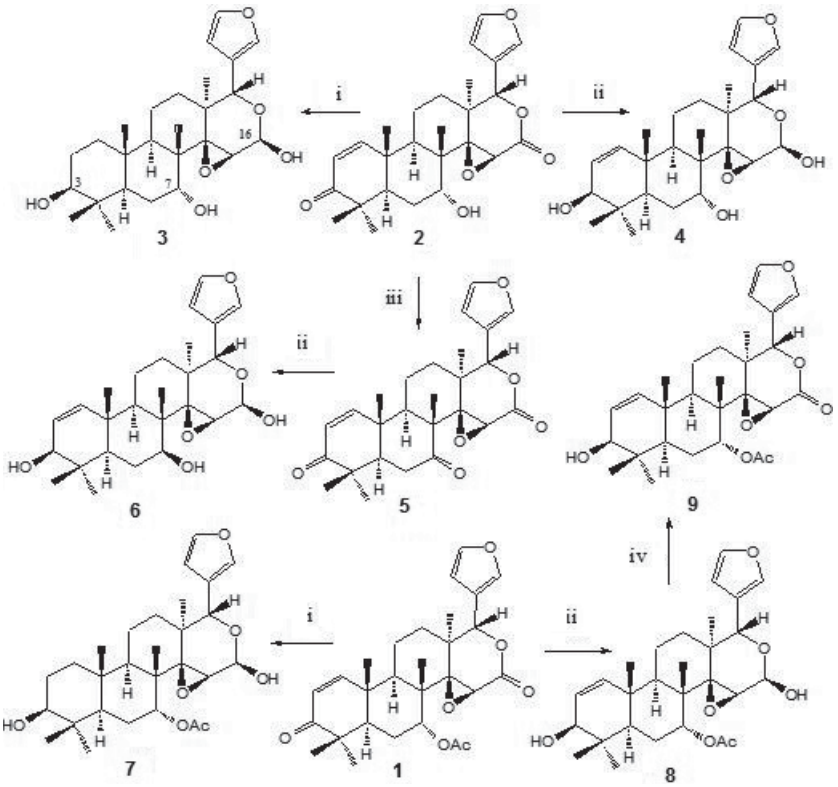
The current authors previously isolated and determined the structure of 5 triterpenoids with an obacunone-type limonoid skeleton[7], 7 triterpenoids with a gedunin-type limonoid skeleton[8], 33 triterpenoids with an apotirucallane skeleton[9,10] and 5 triterpenoids with a tirucallane skeleton[10] from the seeds, leaves, branches, and bark of *C. sinensis*. In addition, gedunin (**1**) molecules have numerous functional groups, so a previous study[11] attempted to introduce fluorine atoms into gedunin-type limonoids via reaction with diethylaminosulfur trifluoride (DAST) in order to determine the chemical reactivity of those groups and to synthesize limonoid analogues not found in nature. Gedunin (**1**) is a gedunin-type limonoid that is isolated from *C. sinensis* in relatively large quantities, and acetylation of that substance yields 7-deacetoxy-7α-hydroxygedunin (**2**). In the current study, those 2 substances were used in reactions (Scheme 1) in order to determine the chemical reactivity

of lactone on the D ring of the 2 substances.

2. Results and Discussion

2.1 Synthesis of analogue **3**

7-deacetoxy-7α-hydroxygedunin (**2**) was reduced with $LiBH_4$ in THF over a period of 1 h at room temperature. This resulted in product **3** at a yield of 86% (Scheme 1). This compound is a colorless, amorphous solid, and HRESIMS indicated that its molecular formula was $C_{26}H_{38}O_6$. Signals for carbons at positions 1-3 of the A ring of this compound were noted at δ 26.3 (CH_2, C-1), 38.5 (CH_2, C-2), and 78.6 (CH, C-3) in the ^{13}C-NMR spectrum. These signals exhibited large upfield shifts in comparison to the signals produced by compound **2**. In addition, signals at δ_H 1.51 (1H, m, H-1α), 1.32 (1H, m, H-1β), 1.56 (1H, m, H-2α), 1.04 (1H, m, H-2β), and 3.29 (1H, m, H-3) were noted in the ^{1}H-NMR spectrum. Signals at the 16 position of the D ring were noted at δ_C 88.2 (CH) and δ_H



Scheme 1. Preparation of **3**-**9**. *Reagents and conditions:* (i) $LiBH_4$, THF, rt, 1 h, 86% for **3**, and 64% for **7**; (ii) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, rt, 1 h, 45% for **4**, 24% for **6**, and 55% for **8**; (iii) TPAP, NMO, 4 Å MS, CH_2Cl_2/CH_3CN, rt, 7 h, 58% for **5**; (iv) MnO_2, CH_2Cl_2/CH_3CN, rt, 2 h, 35% for **9**.

5.29 (1H, dd, J=9.1, 1.3 Hz). An NOE correlation was noted between H–3/H–5, between H–3/H_3–28, between H–15/H–16, and between H–16/H_3–18 in the NOESY spectrum. Thus, this compound was determined to have a structure with 1,4– and 1,2– reduction of the 1–en–3–one structure of the A ring of compound **2** and both the hydroxyl group at position 3 and the hemiacetal hydroxyl group at position 16 in a β configuration.

2.2 Synthesis of analogue **4**

Luche reduction of compound **2** was achieved $NaBH_4$ in methanol in the presence of cerous chloride heptahydrate ($CeCl_3 \cdot 7H_2O$). This reaction took place over a period of 1 h at room temperature and resulted in product **4** at a yield of 45% (Scheme 1). This compound is a colorless, amorphous solid, and HRESIMS indicated that its molecular formula was $C_{26}H_{36}O_6$. A signal for carbon at position 3 of the A ring of this compound was noted at δ 77.2 (CH) in the ^{13}C–NMR spectrum. In addition, signals were noted at δ_H 5.86 (1H, d, J=10.5 Hz, H–1), 5.31 (1H, d, J=10.5 Hz, H–2), and 3.95 (1H, br s, H–3) in the ^{1}H–NMR spectrum. Further 1,2– reduction of the A ring was evident. Signals at position 16 of the D ring were noted at $_C$ 88.1 (CH) and δ_H 5.28 (br s), suggesting the existence of a hemiacetal structure. An NOE correlation between H3–/H–5, between H–3/H_3–28, between H–15/H–16, and between H–16/H_3–18 was indicated in the NOESY spectrum, so both the hydroxyl group at position 3 and the hemiacetal hydroxyl group at position 16 were determined to be in a β configuration. Thus, this compound was determined to have a structure in which the carbonyl groups at positions 3 and 16 of compound **2** were reduced.

2.3 Analogue **5** (7–oxogedunin)

Compound **2** was oxidized with tetrapropylammonium perruthenate (TPAP, $Pr_4N^+ RuO_4^-$) in methylene chloride in the presence of NMO (4–methylmorphorine *N*–oxide) and 4Å molecular sieves, resulting in product **5** at a yield of 58%. Based on HRESIMS findings and analysis of the 2D NMR spectrum, the structure of compound **5** was verified to be that of the known compound 7–oxogedunin[12].

2.4 Synthesis of analogue **6**

Luche reduction of compound **5** was achieved with $NaBH_4$ in methanol in the presence of cerous chloride heptahydrate ($CeCl_3 \cdot 7H_2O$), resulting in product **6** at a yield of 24% (Scheme 1). This compound is a colorless, amorphous solid, and HRESIMS indicated that its molecular formula was $C_{26}H_{36}O_6$. Signals for the carbons at positions 3 and 7 of this compound were noted at δ 77.2 (CH, C–3) and 77.8 (CH, C–7) in the ^{13}C–NMR spectrum, and proton signals were noted at δ_H 3.89 (1H, br s, H–3) and 3.81 (1H, dd, J=10.7, 4.7 Hz, H–7) in the ^{1}H–NMR spectrum. Signals at the 16 position of the D ring were noted at δ_C 88.5 (CH) and δ_H 5.30 (m). An NOE correlation was noted between H–

3/H-5, between H-3/H_3-28, between H-5/H-7, between H-7/H-9, between H-15/H-16, and between H-16/H_3-18 in the NOESY spectrum, so the hydroxyl group at positions 3 and 7 and the hemiacetal hydroxyl group at position 16 were in a β configuration. Thus, the structure of compound **6** was determined.

2.5 Synthesis of analogue **7**

Gedunin (**1**) was reduced with $LiBH_4$ in THF, resulting in product **7** at a yield of 64% (Scheme 1). This compound is a colorless, amorphous solid, and HRESIMS indicated that its molecular formula was $C_{28}H_{40}O_7$. Signals for carbons at positions 1-3 of the A ring of this compound were noted at δ 26.5 (CH_2, C-1), 38.5 (CH_2, C-2), and 78.5 (CH, C-3) in the ^{13}C-NMR spectrum. Signals were noted at δ_H 1.54 (1H, m, H-1α), 1.33 (1H, m, H-1β), 1.69 (1H, m, H-2α), 1.04 (1H, m, H-2β), and 3.27 (1H, m, H-3) in the ^{1}H-NMR spectrum. Signals at the 16 position of the D ring were noted at δ_C 87.9 (CH) and δ_H 5.13 (1H, dd, J=8.8, 1.6 Hz). An NOE correlation was noted between H3-/H-5, between H-3/H_3-28, between H-15/H-16, and between H-16/H_3-18 in the NOESY spectrum, so both the hydroxyl group at position 3 and the hemiacetal hydroxyl group at position 16 were in a β configuration. Thus, the structure of compound **7** was determined.

2.6 Synthesis of analogue **8**

Luche reduction of compound **1** was achieved with $NaBH_4$ in methanol in the presence of cerous chloride heptahydrate ($CeCl_3 \cdot 7H_2O$), resulting in product **8** at a yield of 55% (Scheme 1). This compound is a colorless, amorphous solid, and HRESIMS indicated that its molecular formula was $C_{28}H_{38}O_7$. A signal for carbon at position 3 of the A ring of this compound was noted at δ 77.2 (CH) in the ^{13}C-NMR spectrum. In addition, signals at δ_H 5.87 (1H, dd, J=10.4, 1.8 Hz, H-1), 5.33 (1H, dd, J=10.4, 1.8 Hz, H-2), and 3.91 (1H, br s, H-3) were noted in the ^{1}H-NMR spectrum. Signals at the 16 position of the D ring were noted at δ_C 87.8 (CH) and δ_H 5.13 (1H, d, J=6.0 Hz). An NOE correlation was noted between H3-/H-5, between H-3/H_3-28, between H-15/H-16, and between H-16/H_3-18 in the NOESY spectrum, so both the hydroxyl group at position 3 and the hemiacetal hydroxyl group at position 16 were in a β configuration. Thus, the structure of compound **8** was determined.

2.7 Synthesis of analogue **9** (3β-hydroxygedunin)

Selective oxidation of allyl alcohol in compound **8** was attempted with manganese dioxide (MnO_2) in dichloromethane. This produced compound **9**, which had oxidization only at position 16 of the D ring and not at position 3 of the A ring. Based on HRESIMS findings and the 2D NMR spectrum, compound **9** was verified to be the known compound 3β-hydroxygedunin[13].

As stated above, the lactone-carbonyl group at position 16 of the D ring of a gedunin-type limonoid was readily reduced to a hydroxyl group by $LiBH_4$ or $NaBH_4$ in the presence of cerous chloride. In addition, oxidation of compound **8** with MnO_2 resulted in more ready oxidation of the hemiacetal hydroxyl group at position 16 than of the allyl alcohol at position 3 of the A ring. Although the reduced form had a hemiacetal structure, further reduction failed to result in a 16–methylol form, so this hemiacetal structure is extremely stable.

2.8. Cytotoxicity assay of analogues of gedunin-type limonoids against P–388 murine leukemia cells

The 7 limonoid analogues synthesized in this study were subjected to a cytotoxicity assay using cells from mice with P–388 leukemia (Table 1).

Among the compounds in which the carbonyl group at position 16 of the D ring was oxidized or reduced, compounds **3–8** lacked cytotoxic activity for the most part. Compound **9** had an IC_{50} of 8.3 μg/mL, but this is extremely low in comparison to mitomycin C, which has an IC_{50} of 0.029 μg/mL.

Table 1. Cytotoxic activity of limonoid analogues against P–388 cells *in vitro*

Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
1	3.3	**2**	4.5
3	>10	**4**	>10
5	>10	**6**	>10
7	>10	**8**	>10
9	8.3	mitomycin C	0.029

Experimental

3.1. General

During this experiment, various types of data were obtained using the following equipment.

Melting point: Yanaco MP–3, Optical rotation ($[\alpha]_D$): JASCO P–1030, Mass spectra (MS): Micromass LCT, Infrared (IR) absorption spectra: JASCO FT/IR 620, Ultraviolet (UV) absorption spectra: JASCO V–530, and Nuclear magnetic resonance (NMR) spectra: Bruker AM- 500, DRX- 500, and AV–600. NMR spectra were recorded by and data were processed using the software Bruker XWIN-NMR. In addition, a chemical shift (δ) was expressed in units of ppm. Prydine-d_5 {^{1}H: 7.21 ppm ($C5D_4HN$), ^{13}C: 135.5 ppm}, $CDCl_3$ {^{1}H: 7.26 ppm ($CHCl_3$), ^{13}C: 77.03 ppm}, CD_3OD {^{1}H: 3.31 ppm (CD_2HOD), and ^{13}C: 49.0 ppm} were used as internal standards. High-performance liquid chromatography (HPLC) was performed using the following system. The pump used was the LC–6AD (Shimadzu). Compounds were detected with the SPD–10A UV/vis detector (Shimadzu). The

column used was the Inertsil Prep-ODS (GL Sciences, Inc.) (6 mm i.d. ×250 mm, ODS, 10 μm, 20 mm i.d. ×250 mm, ODS, 10 μm), the Wakosil-II 5C18HG (20 mm i.d. ×250 mm, ODS, 5 μm), or the Capcell Pak C18 UG 80 (10 mm i.d. ×250 mm, ODS, 5 μm). Cytotoxic activity against p-388 murine leukemia cells was measured using methods previously reported[14].

3.2. Reaction procedures

3.2.1 Conversion of compound **2** into compound **3**

Lithium borohydride in THF (lithium borohydride/tetrahydrofuran, $LiBH_4$/THF, ca. 2.0 mol/L, 1 mL) was added to compound **2** (7-deacetoxy-7α-hydroxygedunin, 16 mg, 0.036 mmol), and the resulting mixture was stirred for 1 h at room temperature. The reaction solution was cooled to 0°C, and then the reaction was halted by gradually adding 10% H_2SO_4 (10 drops). The solution was diluted with chloroform (30 mL), and the residue was rinsed with water (15 mL) and a saturated saline solution (15 mL). The solution was dried with anhydrous magnesium sulfate and then filtered. The solvent was removed under reduced pressure, and the resulting residue was purified using ODS HPLC (MeOH/H_2O 60:40), resulting in compound **3** (14 mg, 86%).

Compound **3**: Colorless, amorphous solid; $[\alpha]_D^{27}$ −4.6 (*c* 0.7, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.33 (1H, m, H-21), 7.32 (1H, s, H-23), 6.29 (1H, d, *J*=1.0 Hz, H-22), 5.29 (1H, dd, *J*=9.1, 1.3 Hz, H-16), 5.02 (1H, s, H-17), 3.56 (1H, d, *J*=1.6 Hz, H-15), 3.54 (1H, br s, H-7), 3.29 (1H, m, H-3), 2.17 (1H, dd, *J*=12.1, 6.1 Hz, H-9), 1.81 (1H, m, H-12β), 1.69 (1H, m, H-12α), 1.68 (1H, m, H-11α), 1.66 (2H, m, H-6α and H-6β), 1.56 (1H, m, H-2α), 1.51 (2H, m, H-1α and H-1β), 1.48 (1H, m, H-5), 1.32 (1H, m, H-1β), 1.22 (3H, s, H_3-18), 1.04 (1H, m, H-2β), 0.96 (6H, s, H_3-29 and H_3-30), 0.91 (3H, s, H_3-19), 0.78 (3H, s, H_3-28); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 142.2 (CH, C-23), 140.7 (CH, C-21), 123.3 (C, C-20), 110.4 (CH, C-22), 88.2 (CH, C-16), 78.6 (CH, C-3), 72.7 (CH, C-17), 71.4 (CH, C-7), 68.6 (C, C-14), 60.3 (CH, C-15), 46.9 (CH, C-5), 43.3 (CH, C-9), 43.0 (C, C-8), 38.5 (CH_2, C-2), 38.4 (C, C-10), 37.8 (C, C-13), 36.8 (C, C-4), 27.7 (CH_3, C-28), 27.3 (CH_2, C-6), 26.9 (CH_2, C-12), 26.3 (CH_2, C-1), 18.9 (CH_3, C-18), 18.4 (CH_3, C-30), 16.5 (CH_3, C-19), 15.5 (CH_3, C-29), 15.3 (CH_2, C-11); HRESIMS *m/z* 469.2527 ($[M+Na]^+$, calcd for $C_{26}H_{38}O_6Na$, 469.2566).

3.2.2 Conversion of compound **2** into compound **4**

Compound **2** (31 mg, 0.070 mmol) was dissolved in methanol (1 mL), a catalytic quantity of cerous chloride heptahydrate {cerium(III) chloride heptahydrate} and sodium borohydride (sodium borohydride, $NaBH_4$, 13 mg, 0.34 mmol) were added, and the resulting mixture was stirred for 1 h at room temperature. The reaction solution was cooled to 0°C, and then the reaction was halted by gradually adding 10% H_2SO_4 (10 drops). The solution was diluted with chloroform (30 mL), and the residue was rinsed with water (15 mL) and a saturated saline solution (15 mL). The solution was

dried with anhydrous magnesium sulfate and then filtered. The solvent was removed under reduced pressure, and the resulting residue was purified using ODS HPLC (MeOH/H_2O 60:40), resulting in compound **4** (14 mg, 45%).

Compound **4**: Colorless, amorphous solid; $[\alpha]_D^{27}+14$ (*c* 0.1, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.33 (1H, s, H–23), 7.31 (1H, s, H–21), 6.29 (1H, s, H–22), 5.86 (1H, d, J=10.5 Hz, H–1), 5.31 (1H, d, J=10.5 Hz, H–2), 5.28 (1H, br s, H–16), 5.01 (1H, s, H–17), 3.95 (1H, s, H–3), 3.57 (1H, s, H–15), 3.55 (1H, br s, H–7), 2.33 (1H, dd, J=12.7, 5.9 Hz, H–9), 1.97 (1H, m, H–5), 1.85 (1H, m, H–6α), 1.84 (1H, m, H–11α), 1.72 (1H, m, H–6β), 1.65 (1H, m, H–11β), 1.52 (1H, m, H–12β), 1.38 (1H, m, H–12α), 1.19 (3H, s, H_3–18), 1.07 (3H, s, H_3–19), 0.98 (3H, s, H_3–30), 0.97 (3H, s, H_3–28), 0.83 (3H, s, H_3–29); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 142.2 (CH, C–23), 140.7 (CH, C–21), 137.6 (CH, C–1), 125.9 (CH, C–2), 123.2 (C, C–20), 110.3 (CH, C–22), 88.1 (CH, C–16), 77.2 (CH, C–3), 72.7 (CH, C–17), 71.2 (CH, C–7), 70.0 (C–14), 60.3 (CH, C–15), 44.7 (CH, C–5), 43.8 (C, C–8), 40.6 (CH, C–9), 39.7 (C, C–10), 36.7 (2C, C, C–4 and C–13), 27.4 (CH_3, C–28), 26.7 (CH_2, C–6), 26.4 (CH_2, C–12), 19.7 (CH_3, C–19), 19.1 (CH_3, C–18), 18.7 (CH_3, C–30), 17.5 (CH_3, C–29), 15.7 (CH_2, C–11); HRESIMS *m/z* 427.2480 ($[M-OH]^+$, calcd for $C_{26}H_{35}O5$, 427.2484).

3.2.3 Conversion of compound **2** into compound **5**

Compound **2** (33 mg, 0.075 mmol) was dissolved in dichloromethane/acetonitrile (10: 1, 2 mL), 4–methylmorphorine *N*-oxide (NMO, 13 mg, 0.11 mmol), 4Å molecular sieves (38 mg), and tetrapropylammonium perruthenate (TPAP, $Pr_4N^+RuO_4^-$, 2.6 mg, 0.0074 mmol) were added, and the resulting mixture was stirred for 7 h at room temperature in an argon atmosphere. The reaction solution was diluted with chloroform (30 mL), and the residue was rinsed with a saturated sodium sulfite solution (10 mL) and saturated saline solution (10 mL). The solution was dried with anhydrous sodium sulfate and then filtered. The solvent was removed under reduced pressure, and the resulting residue was purified using ODS HPLC (MeOH/H_2O 60:40), resulting in compound **5** (19 mg, 58%). The ^{1}H-NMR spectrum and HRESIMS for this this compound agreed with previously reported findings for 7–oxogedunin[12].

Compound **5** (7–oxogedunin): Colorless, amorphous solid; $[\alpha]_D^{27}-56$(*c* 0.3, $CHCl_3$); HRESIMS *m/z* 439.2104 ($[M+H]^+$, calcd for $C_{26}H_{31}O_6$, 439.2121).

3.2.4 Conversion of compound **5** into compound **6**

Compound **5** (7–oxogedunin, 19 mg, 0.043 mmol) was dissolved in methanol (1 mL), a catalytic amount of cerous chloride heptahydrate and sodium borohydride (7.8 mg, 0.21 mmol) were added, and the resulting mixture was stirred for 1 h at room temperature. The reaction solution was cooled

to 0°C, and then the reaction was halted by gradually adding 10% H_2SO_4 (10 drops). The solution was diluted with chloroform (30 mL), and the residue was rinsed with water (15 mL) and a saturated saline solution (15 mL). The solution was dried with anhydrous magnesium sulfate and then filtered. The solvent was removed under reduced pressure, and the resulting residue was purified using ODS HPLC (MeOH/H_2O 60:40), resulting in compound **6** (4.7 mg, 24%).

Compound **6**: Colorless, amorphous solid; $[\alpha]_D^{27}$ + 57 (*c* 0.2, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) 7.33 (2H, m, H–21 and H–23), 6.29 (1H, s, H–22), 5.83 (1H, dd, *J*=10.3, 1.4 Hz, H–1), 5.34 (1H, dd, *J*=10.3, 1.4 Hz, H–2), 5.30 (1H, m, H–16), 5.00 (1H, br s, H–17), 4.09 (1H, d, *J*=1.5 Hz, H–15), 3.89 (1H, br s, H–3), 3.81 (1H, dd, *J*=10.7, 4.7 Hz, H–7), 1.88 (1H, m, H–9), 1.85 (1H, m, H–6α), 1.81 (1H, m, H–11β), 1.72 (1H, m, H–11α), 1.63 (1H, m, H–12β), 1.59 (1H, m, H–6β), 1.34 (1H, m, H–5), 1.31 (1H, m, H–12α), 1.18 (3H, s, H_3–18), 1.06 (3H, s, H_3–19), 1.02 (3H, s, H_3–30), 1.01 (3H, s, H_3–28), 0.83 (3H, s, H_3–29); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 142.3 (CH, C–23), 140.7 (CH, C–21), 137.0 (CH, C–1), 126.6 (CH, C–2), 123.1 (C, C–20), 110.2 (CH, C–22), 88.5 (CH, C–16), 77.8 (CH, C–7), 77.2 (CH, C–3), 72.7 (CH, C–17), 72.2 (C, C–14), 58.9 (CH, C–15), 51.3 (CH, C–5), 46.5 (CH, C–9), 45.2 (C, C–8), 39.3 (C, C–10), 37.5 (C, C–13), 37.1 (C, C–4), 28.6 (CH_2, C–6), 28.2 (CH_2, C–12), 27.4 (CH_3, C–28), 21.0 (CH_3, C–18), 19.4 (CH_3, C–19), 17.3 (CH_3, C–29), 16.8 (CH_2, C–11), 13.1 (CH_3, C–30); HRESIMS *m/z* 467.2402 ($[M + Na]^+$, calcd for $C_{26}H_{36}O_6Na$, 467.2410).

3.2.5 Conversion of compound **1** into compound **7**

Lithium borohydride in THF (1 mL) was added to compound **1** (gedunin, 20 mg, 0.041 mmol), and the resulting mixture was stirred for 1 h at room temperature. The reaction solution was cooled to 0°C, and then the reaction was halted by gradually adding 10% H_2SO_4 (10 drops). The solution was diluted with chloroform (30 mL), and the residue was rinsed with water (15 mL) and a saturated saline solution (15 mL). The solution was dried with anhydrous magnesium sulfate and then filtered. The solvent was removed under reduced pressure, and the resulting residue was purified using ODS HPLC (MeOH/H_2O 60:40), resulting in compound **7** (13 mg, 64%).

Compound **7**: Colorless, amorphous solid; $[\alpha]_D^{27}$ −21 (*c* 0.07, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.34 (1H, s, H–23), 7.30 (1H, m, H–21), 6.29 (1H, s, H–22), 5.13 (1H, dd, *J*=8.8, 1.6 Hz, H–16), 4.99 (1H, s, H–17), 4.65 (1H, br s, H–7), 3.30 (1H, d, *J*=1.6 Hz, H–15), 3.27 (1H, m, H–3), 2.21 (1H, m, H–9), 2.12 (3H, s, OAc–7), 1.84 (1H, m, H–6α), 1.72 (1H, m, H–6β), 1.70 (1H, m, H–12β), 1.69 (1H, m, H–2α), 1.68 (1H, m, H–11α), 1.62 (1H, m, H–12α), 1.54 (1H, m, H–1β), 1.53 (1H, m, H–11β), 1.33 (1H, s, H–1β), 1.32 (1H, m, H–5), 1.24 (3H, s, H_3–18), 1.04 (1H, m, H–2β), 1.01 (3H, s, H_3–30), 0.92 (3H, s, H_3–19), 0.88 (3H, s, H_3–28), 0.76 (3H, s, H_3–29); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.9 (C, O*C*OCH_3–7), 142.3 (CH, C–23), 140.7 (CH, C–21), 123.1 (CH, C–20), 110.3 (CH, C–22), 87.9 (CH, C–16), 78.5 (CH,

C–3), 74.9 (CH, C–7), 72.5 (CH, C–17), 69.7 (C, C–14), 58.7 (CH, C–15), 48.2 (CH, C–5), 44.8 (CH, C–9), 44.4 (C, C–8), 38.5 (CH_2, C–2), 38.3 (C, C–10), 37.5 (C, C–13), 36.8 (C, C–4), 27.7 (CH_3, C–28), 27.2 (CH_2, C–12), 26.5 (CH_2, C–1), 23.4 (CH_2, C–6), 21.4 (CH_3, OCO*C*H_3–7), 19.3 (CH_3, C–18), 18.0 (CH_3, C–30), 16.5 (CH_3, C–19), 15.2 (CH_2, C–11), 15.2 (CH_3, C–29); HRESIMS *m/z* 471.2734 ($[M-OH]^+$, calcd for $C_{28}H_{39}O_6$, 471.2747).

3.2.6 Conversion of compound **1** into compound **8**

Compound **1** (58 mg, 0.12 mmol) was dissolved in methanol (1 mL), a catalytic quantity of cerous chloride heptahydrate and sodium borohydride (23 mg, 0.61 mmol) were added, and the resulting mixture was stirred for 1 h at room temperature. The reaction solution was cooled to 0°C, and then the reaction was halted by gradually adding 10% H_2SO_4 (10 drops). The solution was diluted with chloroform (30 mL), and the residue was rinsed with water (15 mL) and a saturated saline solution (15 mL). The solution was dried with anhydrous magnesium sulfate and then filtered. The solvent was removed under reduced pressure, and the resulting residue was purified using ODS HPLC (MeOH/H_2O 60:40), resulting in compound **8** (32 mg, 55%).

Compound **8**: Colorless, amorphous solid; $[\alpha]_D^{27}$ −23 (*c* 0.2, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.33 (2H, m, H–21 and H–23), 6.27 (1H, s, H–22), 5.87 (1H, dd, J=10.4, 1.8 Hz, H–1), 5.33 (1H, dd, J=10.4, 1.8 Hz, H–2), 5.13 (1H, d, J=6.0 Hz, H–16), 4.98 (1H, br s, H–17), 4.65 (1H, d, J=1.8 Hz, H–7), 3.91 (1H, br s, H–3), 3.18 (1H, d, J=1.6 Hz, H–15), 2.36 (1H, dd, J=12.8, 5.3 Hz, H–9), 2.11 (3H, s, OAc–7), 1.89 (1H, m, H–6α), 1.85 (1H, m, H–11α), 1.78 (1H, m, H–5), 1.73 (1H, m, H–6β), 1.64 (1H, m, H–11β), 1.52 (1H, m, H–12β), 1.37 (1H, m, H–12α), 1.22 (3H, s, H_3–18), 1.08 (3H, s, H_3–19), 1.02 (3H, s, H_3–30), 0.89 (3H, s, H_3–28), 0.81 (3H, s, H_3–29); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.9 (C, OCOCH_3–7), 142.3 (CH, C–23), 140.7 (CH, C–21), 137.3 (CH, C–1), 126.1 (CH, C–2), 123.0 (C, C–20), 110.2 (CH, C–22), 87.8 (CH, C–16), 77.2 (CH, C–3), 74.5 (CH, C–7), 72.5 (CH, C–17), 69.4 (C, C–14), 58.6 (CH, C–15), 46.1 (CH, C–5), 43.0 (C, C–8), 42.0 (CH, C–9), 39.5 (C, C–10), 36.8 (C, C–13), 36.6 (C, C–4), 27.5 (CH_3, C–28), 26.7 (CH_2, C–12), 23.2 (CH_2, C–6), 21.4 (CH_3, OCO*C*H_3–7), 19.7 (CH_3, C–19), 19.5 (CH_3, C–18), 18.2 (CH_3, C–30), 17.1 (CH_3, C–29), 15.6 (CH_2, C–11); HRESIMS *m/z* 469.2573 ($[M-OH]^+$, calcd for $C_{28}H_{37}O_6$, 469.2590).

3.2.7 Conversion of compound **8** into compound **9**

Compound **8** (37 mg, 0.077 mmol) was dissolved in dichloromethane (2 mL), manganese dioxide (MnO_2, 198 mg, 2.3 mmol) was added, and the resulting mixture was stirred for 2 h at room temperature. The reaction solution was diluted with chloroform (30 mL) and filtered. The solvent was then removed under reduced pressure, and the resulting residue was purified using ODS HPLC (MeOH/H_2O 65: 35), resulting in compound **9** (13 mg, 35%). The ^{1}H-NMR spectrum and HRESIMS

results for this this compound agreed with previously reported findings for 3β-hydroxygedunin[13].

Compound **9** (3β-hydroxygedunin): Colorless, amorphous solid; $[\alpha]_D^{27}$ + 31 (*c* 0.6, $CHCl_3$); HRESIMS *m/z* 485.2536 ($[M + H]^+$, calcd for $C_{28}H_{37}O_7$, 485.2539).

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