名古屋学院大学論集　人文・自然科学篇　第 55 巻　第 1 号　pp. 25–33

〔Article〕

The structure-activity relationship in a Gedunin-type Limonoid (7–Deacetoxy–7α –hydroxygedunin) with a modified functional group at the 7–position

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Abstract

Chinese toon, or *Cedrela sinensis* Juss. (Meliaceae), is a broad-leafed tree found in China and South Korea. Gedunin (**1**)[1] is a limonoid that is abundant in plants in the Meliaceae family. Gedunin (**1**) is reported to have antimalarial activity comparable to that of quinine[2]. However, gedunin-type limonoid molecules have numerous functional groups and the reactivity of those groups is not known, so few studies have chemically modified gedunin-type limonoids. Studies have examined the structure-activity relationship of ring B of gedunin-type limonoids by comparing similar natural limonoids. Those studies found that the functional group at the 7–position affects whether gedunin-type limonoids have antimalarial activity[2-4] or whether they have biological activity, e.g. inhibiting insect growth[5] or intake[6]. Gedunin (**1**) has an acetyl group at the 7–position and it has potent antimalarial activity. When gedunin is acetylated (**2**), however, that activity is attenuated[2]. In comparison to gedunin (**1**), its deacetylated form (**2**) has somewhat greater inhibition of growth and intake[5]. Very few previous studies have compared the anti-tumor activity of limonoid analogues with a chemically modified ring B. Thus, the current study used 7–deacetoxy–7α –hydroxygedunin (**2**) to obtain new findings regarding structure and activity relationships with anti-tumor activity. This study synthesized 6 new analogues (**3**-**8**) by substituting an acyl group for the acetyl group at the 7–position on ring B, and this study examined their cytotoxic activity against murine P–388 leukemia cells. Compounds with an acyl group in place of an acetyl group at the 7–position on ring B had more potent activity if they had more chlorine atoms.

Keywords: Gedunin-type limonoids, 7–Deacetoxy–7α –hydroxygedunin, Acyl substitution, P–388 murine leukemia cells

発行日　2018年7月31日

Gedunin型リモノイド7–deacetoxy–7α–hydroxygedunin の 7位に修飾したアナログの構造活性相関について

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1. Introduction

We 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-type skeleton[9, 10], and 5 triterpenoids with a tirucallane skeleton[10] from the seeds, leaves, branches, and bark of *Cedrela sinensis* Juss. (Meliaceae). The known compound gedunin (**1**) can be obtained in relative abundance from that plant. A study has reported that gedunin (**1**) has *in vitro* activity against the chloroquine-resistant K1 strain of *Plasmodium falciparum*[3]. Studies on the structure-activity relationship of gedunin-type limonoids[2-6] have compared similar natural limonoids and they have synthesized derivatives by replacing the acetyl group at the 7–position and reducing the acetyl group at the 1– and 2–positions. MacKinnon et al. examined the structure-activity relationship of compounds related to gedunin (**1**), and they indicated that elements such as the double bond between the acetyl groups at the 1– and 2–positions on ring A, the acetyl group at the 7–position on ring B, epoxides at the 14– and 15–position on ring D, and the furan ring in the side chain were crucial to the display of antimalarial activity[5]. These findings were all based on assessment of the activity of derivatives that were easily obtained from gedunin (**1**).

Gedunin molecules have a number of functional groups, so we previously attempted to introduce fluorine atoms into gedunin-type limonoids through fluorination with diethylaminosulfur trifluoride (DAST) in order to determine the chemical reactivity of those limonoids and to synthesize limonoid analogues not found in nature[11]. In order to determine the effects of the acetyl group at the 7–position in gedunin, the current study chemically transformed 7–deacetoxy–7α–hydroxygedunin (**2**) through acylation and synthesized 6 analogues. The starting compound gedunin (**1**) is relatively abundant in the bark of *C. sinensis*, so it can readily be obtained. Gedunin can be deacetylated to obtain 7–deacetoxy–7α–hydroxygedunin (**2**).

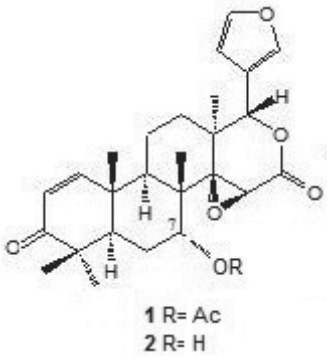


Fig. 1. Structures of gedunin (**1**) and 7–deacetoxy–7α–hydroxygedunin (**2**).

2. Results and Discussion

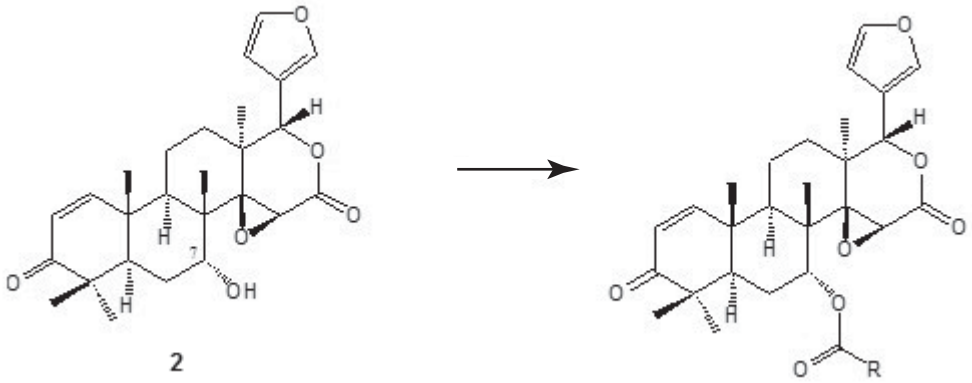
7–deacetoxy–7α–hydroxygedunin (**2**) was allowed to react with di-*tert*-butyldicarbonate {$(COO\text{-}t\text{-}Bu)_2O$} in pyridine for 24 h at room temperature in the presence of 4–dimethylaminopyridine (DMAP). This resulted in product **3**, with a yield of 53% (Scheme 1). According to HRESIMS, this compound had a molecular formula of $C_{31}H_{40}O_8$, and a signal due to a *tert*-butyloxycarbonyl group {δ_H 1.49 (s, 9H), δ_C 152.7 (C, C–1'), 83.0 (C, C–2'), 27.7 (3C, CH_3, C–3')} was noted in ^{1}H- and ^{13}C-NMR spectra.

Similarly, compound **2** was allowed to react with propionic anhydride {$(CH_3CH_2CO)_2O$} in pyridine for 24 h at room temperature in the presence of DMAP. This resulted in product **4**, with a yield of 86% (Scheme 1). According to HRESIMS, the compound had a molecular formula of $C_{29}H_{36}O_7$, and signals due to a propionyl group {δ_H 2.35 (2H, m), 1.19 (3H, m), δ_C 173.4 (C, C–1'), 28.0 (CH_2, C–2'), 9.2 (CH_3, C–3')} were noted in ^{1}H- and ^{13}C-NMR spectra.

Similarly, compound **2** was allowed to react with butyric anhydride {$[CH_3(CH_2)_2CO]_2O$} in pyridine for 24 h at room temperature in the presence of DMAP. This resulted in product **5**, with a yield of 93% (Scheme 1). According to HRESIMS, the compound had a molecular formula of $C_{30}H_{38}O_7$, and signals due to a butyryl group {δ_H 2.30 (2H, m), 1.67 (2H, m), 0.96 (3H, t, J=7.4 Hz), δ_C 172.6 (C, C–1'), 36.4 (CH_2, C–2'), 18.4 (CH_2, C–3'), 13.8 (CH_3, C–4')} were noted in ^{1}H- and ^{13}C-NMR spectra.

Compound **2** was allowed to react with monochloroacetic acid anhydride {chloroacetic anhydride, $(CH_2ClCO)_2O$} in dichloromethane for 72 h at room temperature in the presence of DMAP. This resulted in product **6**, with a yield of 66% (Scheme 1). According to HRESIMS, this compound had a molecular formula of $C_{28}H_{33}O_7Cl$, and a signal due to a chloroacetyl group {δ_H 4.06 (2H, d, J=1.8 Hz), δ_C 166.3 (C, C–1'), 40.6 (CH_2, C–2')} was noted in ^{1}H- and ^{13}C-NMR spectra.

Similarly, compound **2** was allowed to react with dichloroacetic anhydride {$(CHCl_2CO)_2O$} in dichloromethane for 72 h at room temperature in the presence of DMAP. This resulted in product **7**,



Scheme 1. Acylation of compound **2**.[a]

Compound	R	Acylating agent	Solvent/Catalyst (equiv)	Time (h)	Yield (%)
3	*t*-BuO	$(COO\text{-}t\text{-}Bu)_2O$	Pyridine/DMAP (0.25)	24	53
4	Et	$(CH_3CH_2CO)_2O$	Pyridine/DMAP (0.25)	24	86
5	*n*-Pr	$[CH_3(CH_2)_2CO]_2O$	Pyridine/DMAP (0.25)	24	93
6	CH_2Cl	$(CH_2ClCO)_2O$	CH_2Cl_2/DMAP (0.5)	72	66
7	$CHCl_2$	$(CHCl_2CO)_2O$	CH_2Cl_2/DMAP (0.5)	72	53
8	CCl_3	$(CCl_3CO)_2O$	CH_2Cl_2/DMAP (0.5)	72	51

[a] Reactions were carried out using 10 equiv of an acylating agent at room temperature.

with a yield of 53% (Scheme 1). According to HRESIMS, this compound had a molecular formula of $C_{28}H_{32}O_7Cl_2$, and a signal due to a dichloroacetyl group {δ_H 5.96 (s), δ_C 163.3 (C, C–1'), 64.1 (CH, C–2')} was noted in ^{1}H- and ^{13}C-NMR spectra.

Similarly, compound **2** was allowed to react with trichloroacetic anhydride {$(CCl_3CO)_2O$} in dichloromethane for 72 h at room temperature in the presence of DMAP. This resulted in product **8**, with a yield of 51% (Scheme 1). According to HRESIMS, this compound had a molecular formula of $C_{28}H_{31}O_7Cl_3$, and a signal due to a trichloroacetyl group {δ_C 160.9 (C, C–1'), 90.4 (C, C–2')} was noted in the ^{13}C-NMR spectrum.

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

In analogues **3**–**8**, an acyl group was transferred to the hydroxyl group at the 7–position, all of the analogues has more potent activity than that of compounds **1** and **2**. Among these derivatives, compound **8** (which had a trichloroacetyl group) had 21 times the activity of compound **1**. As findings of the structure-activity relationship by chemical modification of the gedunin-type limonoids, all of the derivatives had weaker cytotoxic activity than the IC_{50} value of 0.029 μg/mL of mitomycin C. Furthermore, compounds with an acyl group in place of an acetyl group at the 7–position on ring B had more potent cytotoxic activity if they had more chlorine atoms.

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

Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
1	3.3	**2**	4.5
3	2.3	**4**	1.9
5	0.73	**6**	0.54
7	0.44	**8**	0.16
		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. A chemical shift (δ) was expressed in units of ppm. Prydine-d_5 {^{1}H: 7.21 ppm (C_5D_4HN), ^{13}C: 135.5 ppm}, $CDCl_3$ {^{1}H: 7.26 ppm ($CHCl_3$), ^{13}C: 77.03 ppm}, and CD_3OD {^{1}H: 3.31 ppm (CD_2HOD), ^{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 murine P–388 leukemia cells was measured using methods previously reported [12].

3.2. Reaction procedures

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

Compound **2** (7–deacetoxy–7–hydroxygedunin, 10 mg, 0.023 mmol) was dissolved in pyridine (1 mL), 4–dimethylaminopyridine (DMAP, 0.7 mg, 0.0057 mmol) and di-*tert*-butyldicarbonate {$(COO\text{-}t\text{-}Bu)_2O$, 48 μL, 0.22 mmol} were added, and the resulting mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure and the resulting residue was purified using ODS HPLC ($MeOH/H_2O$ 70:30), resulting in compound **3** (6.5 mg, 53%).

Compound **3**: Colorless, amorphous solid; $[\alpha]_D^{28}$ +42 (*c* 0.7, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ

7.40 (1H, s, H–21), 7.40 (1H, m, H–23), 7.08 (1H, d, J=10.2 Hz, H–1), 6.35 (1H, d, J=0.86 Hz, H–22), 5.85 (1H, d, J=10.2 Hz, H–2), 5.58 (1H, s, H–17), 4.34 (1H, d, J=1.4 Hz, H–7), 3.59 (1H, s, H–15), 2.49 (1H, dd, J=12.7, 5.9 Hz, H–9), 2.30 (1H, dd, J=13.3, 2.3 Hz, H–5), 1.96 (1H, m, H–11), 1.93 (1H, m, H–6), 1.83 (1H, m, H–11), 1.80 (1H, m, H–6), 1.70 (1H, m, H–12), 1.59 (1H, m, H–12), 1.49 (9H, s, OCO-*t*-Bu), 1.24 (3H, s, H_3–18), 1.21 (3H, s, H_3–19), 1.10 (3H, s, H_3–30), 1.09 (3H, s, H_3–28), 1.08 (3H, s, H_3–29); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 204.1 (C, C–3), 167.0 (C, C–16), 157.1 (CH, C–1), 152.7 (C, C–1'), 143.0 (CH, C–23), 141.2 (CH, C–21), 126.0 (CH, C–2), 120.6 (C, C–20), 110.0 (CH, C–22), 83.0 (C, C–2'), 78.2 (CH, C–17), 75.2 (CH, C–7), 69.8 (C, C–14), 56.9 (CH, C–15), 45.5 (CH, C–5), 44.1 (C, C–4), 42.9 (C, C–8), 40.0 (C, C–10), 39.2 (CH, C–9), 38.5 (C, C–13), 27.7 (3C, CH_3, C–3'), 26.9 (CH_3, C–29), 26.2 (CH_2, C–12), 23.3 (CH_2, C–6), 21.3 (CH_3, C–28), 19.8 (CH_3, C–30), 18.3 (CH_3, C–18), 17.7 (CH_3, C–19), 15.0 (CH_2, C–11); HRESIMS m/z 563.2600 ($[M+Na]^+$, calcd for $C_{31}H_{40}O_8Na$, 563.2621).

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

Compound **2** (7.6 mg, 0.017 mmol) was dissolved in pyridine (1 mL), DMAP (0.5 mg, 0.0041 mmol) and propionic anhydride {$(CH_3CH_2CO)_2O$, 23 mg, 0.18 mmol} were added, and the resulting mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure and the resulting residue was purified using ODS HPLC (MeOH/H_2O 70:30), resulting in compound **4** (7.4 mg, 86%).

Compound **4**: Colorless, amorphous solid; $[\alpha]_D^{27}$+37 (c 0.3, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.41 (1H, s, H–21), 7.41 (1H, d, J=1.1 Hz, H–23), 7.09 (1H, d, J=10.2 Hz, H–1), 6.34 (1H, s, H–22), 5.86 (1H, d, J=10.2 Hz, H–2), 5.60 (1H, s, H–17), 4.58 (1H, br s, H–7), 3.53 (1H, s, H–15), 2.49 (1H, dd, J=12.7, 6.3 Hz, H–9), 2.35 (2H, m, H–2'), 2.17 (1H, dd, J=13.2, 2.2 Hz, H–5), 2.00 (1H, m, H–11), 1.91 (1H, m, H–6), 1.83 (1H, m, H–6), 1.82 (1H, m, H–11), 1.73 (1H, m, H–12), 1.60 (1H, m, H–12), 1.24 (3H, s, H_3–18), 1.22 (3H, s, H_3–19), 1.19 (3H, m, H–3'), 1.16 (3H, s, H_3–30), 1.07 (3H, s, H_3–29), 1.05 (3H, s, H_3–28); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 204.0 (C, C–3), 173.4 (C, C–1'), 167.4 (C, C–16), 157.0 (CH, C–1), 143.1 (CH, C–23), 141.2 (CH, C–21), 126.0 (CH, C–2), 120.5 (C, C–20), 109.9 (CH, C–22), 78.2 (CH, C–17), 72.8 (CH, C–7), 69.7 (C, C–14), 57.1 (CH, C–15), 46.1 (CH, C–5), 44.1 (C, C–4), 42.7 (C, C–8), 40.1 (C, C–10), 39.6 (CH, C–9), 38.8 (C, C–13), 28.0 (CH_2, C–2'), 27.2 (CH_3, C–28), 26.0 (CH_2, C–12), 23.3 (CH_2, C–6), 21.2 (CH_3, C–29), 19.8 (CH_3, C–19), 18.4 (CH_3, C–30), 17.8 (CH_3, C–18), 15.0 (CH_2, C–11), 9.2 (CH_2, C–3'); HRESIMS m/z 497.2520 ($[M+H]^+$, calcd for $C_{29}H_{37}O_7$, 497.2539).

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

Compound **2** (12 mg, 0.028 mmol) was dissolved in pyridine (1 mL), DMAP (0.9 mg, 0.0074 mmol) and butyric anhydride {$[CH_3(CH_2)_2CO]_2O$, 44 mg, 0.28 mmol} were added, and the resulting mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure and the

resulting residue was purified using ODS HPLC (MeOH/H_2O 70:30), resulting in compound **5** (13 mg, 93%).

Compound **5**: Colorless, amorphous solid; $[\alpha]_D^{27}$+42 (*c* 0.6, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.41 (2H, s, H–21 and H–23), 7.09 (1H, d, *J*=10.2 Hz, H–1), 6.34 (1H, s, H–22), 5.86 (1H, d, *J*=10.2 Hz, H–2), 5.60 (1H, s, H–17), 4.57 (1H, br s, H–7), 3.52 (1H, s, H–15), 2.49 (1H, dd, J=12.7, 6.2 Hz, H–9), 2.30 (2H, m, H–2'), 2.16 (1H, dd, *J*=13.2, 2.1 Hz, H–5), 2.01 (1H, m, H–11), 1.92 (1H, m, H–6), 1.83 (1H, m, H–11), 1.81 (1H, m, H–6), 1.74 (1H, m, H–12), 1.67 (2H, m, H–3'), 1.60 (1H, m, H–12), 1.24 (3H, s, H_3–18), 1.22 (3H, s, H_3–19), 1.16 (3H, s, H_3–30), 1.07 (3H, s, H_3–29), 1.05 (3H, s, H_3–28), 0.96 (3H, t, *J*=7.4 Hz, H–4'); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 204.0 (C, C–3), 172.6 (C, C–1'), 167.3 (C, C–16), 157.0 (CH, C–1), 143.1 (CH, C–23), 141.2 (CH, C–21), 126.0 (CH, C–2), 120.5 (C, C–20), 109.9 (CH, C–22), 78.2 (CH, C–17), 72.8 (CH, C–7), 69.7 (C, C–14), 57.0 (CH, C–15), 46.0 (CH, C–5), 44.1 (C, C–4), 42.6 (C, C–8), 40.1 (C, C–10), 39.5 (CH, C–9), 38.7 (C, C–13), 36.4 (CH_2, C–2'), 27.2 (CH_3, C–28), 26.0 (CH_2, C–12), 23.3 (CH_2, C–6), 21.2 (CH_3, C–29), 19.8 (CH_3, C–19), 18.4 (CH_3, C–30), 18.4 (CH_2, C–3'), 17.8 (CH_3, C–18), 15.0 (CH_2, C–11), 13.8 (CH_3, C–4'); HRESIMS *m/z* 511.2707 ($[M+H]^+$, calcd for $C_{30}H_{39}O_7$, 511.2696).

3.2.4 Conversion of compound **2** into compound **6**

Compound **2** (13 mg, 0.030 mmol) was dissolved in dichloromethane (2 mL), DMAP (1.8 mg, 0.015 mmol) and monochloroacetic acid anhydride {chloroacetic anhydride, $(CH_2ClCO)_2O$, 50 mg, 0.29 mmol} were added, and the resulting mixture was stirred for 72 h at room temperature. The solvent was removed under reduced pressure and the resulting residue was purified using ODS HPLC (MeOH/H_2O 65:35), resulting in compound **6** (9.3 mg, 66%).

Compound **6**: Colorless, amorphous solid; $[\alpha]_D^{27}$+44 (*c* 0.5, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.41 (1H, s, H–21), 7.41 (1H, d, *J*=1.2 Hz, H–23), 7.09 (1H, d, *J*=10.2 Hz, H–1), 6.34 (1H, s, H–22), 5.87 (1H, d, *J*=10.2 Hz, H–2), 5.62 (1H, s, H–17), 4.64 (1H, d, *J*=1.2 Hz, H–7), 4.06 (2H, d, *J*=1.8 Hz, H–2'), 3.51 (1H, m, H–15), 2.52 (1H, dd, *J*=12.7, 6.3 Hz, H–9), 2.21 (1H, dd, *J*=13.3, 2.3 Hz, H–5), 2.02 (1H, m H–6), 2.00 (1H, m, H–11), 1.87 (1H, m, H–6), 1.85 (1H, m, H–11), 1.73 (1H, m, H–12β), 1.62 (1H, m, H–12), 1.26 (3H, s, H_3–18), 1.23 (3H, s, H_3–19), 1.12 (3H, s, H_3–30), 1.08 (6H, s, H_3–28 and H_3–29); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 203.8 (C, C–3), 167.3 (C, C–16), 166.3 (C, C–1'), 156.7 (CH, C–1), 143.1 (CH, C–23), 141.2 (CH, C–21), 126.1 (CH, C–2), 120.3 (C, C–20), 109.9 (CH, C–22), 78.3 (CH, C–17), 75.2 (CH, C–7), 69.7 (C, C–14), 57.1 (CH, C–15), 45.9 (CH, C–5), 44.0 (C, C–4), 42.7 (C, C–8), 40.6 (CH_2, C–2'), 40.0 (C, C–10), C–9), 39.5 (CH, C–9), 38.9 (C, C–13), 27.3 (CH_3, C–28), 25.9 (CH_2, C–12), 23.2 (CH_2, C–6), 21.2 (CH_3, C–29), 19.8 (CH_3, C–19), 18.4 (CH_3, C–30), 17.8 (CH_3, C–18), 15.0 (CH_2, C–11); HRESIMS *m/z* 517.1979 ($[M+H]^+$, calcd for $C_{28}H_{34}O_7Cl$, 517.1993).

3.2.5 Conversion of compound **2** into compound **7**

Compound **2** (12 mg, 0.028 mmol) was dissolved in dichloromethane (2 mL), DMAP (1.7 mg, 0.014 mmol) and dichloroacetic anhydride {$(CHCl_2CO)_2O$, 48 μL, 0.27 mmol} were added, and the resulting mixture was stirred for 72 h at room temperature. The solvent was removed under reduced pressure and the resulting residue was purified using ODS HPLC (MeOH/H_2O 65:35), resulting in compound **7** (7.8 mg, 53%).

Compound **7**: Colorless, amorphous solid; $[\alpha]_D^{27}$ + 28 (*c* 0.4, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.41 (2H, s, H–21 and H–23), 7.10 (1H, d, *J*=8.6 Hz, H–1), 6.34 (1H, s, H–22), 5.96 (1H, s, H–2'), 5.87 (1H, d, *J*=8.6 Hz, H–2), 5.58 (1H, s, H–17), 4.65 (1H, br s, H–7), 3.53 (1H, s, H–15), 2.57 (1H, m, H–9), 2.27 (1H, d-like, *J*=3.2 Hz, H–5), 2.07 (1H, m, H–6), 2.04 (1H, m, H–11), 1.88 (1H, m, H–6), 1.85 (1H, m, H–11), 1.73 (1H, m, H–12), 1.61 (1H, m, H–12), 1.25 (3H, s, H_3–18), 1.24 (3H, s, H_3–19), 1.23 (3H, s, H_3–30), 1,08 (3H, s, H_3–29), 1.07 (3H, s, H_3–28); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 203.8 (C, C–3), 166.6 (C, C–16), 163.3 (C, C–1'), 156.8 (CH, C–1), 143.1 (CH, C–23), 141.3 (CH, C–21), 126.2 (CH, C–2), 120.3 (C, C–20), 109.9 (CH, C–22), 78.1 (CH, C–17), 76.4 (CH, C–7), 69.4 (C, C–14), 64.1 (CH, C–2'), 57.5 (CH, C–15), 45.6 (CH, C–5), 44.1 (C, C–4), 42.8 (C, C–8), 40.1 (C, C–10), 39.2 (C, C–9), 38.7 (C, C–13), 27.4 (CH_3, C–28), 25.8 (CH_2, C–12), 22.8 (CH_2, C–6), 21.3 (CH_3, C–29), 20.0 (CH_3, C–19), 18.6 (CH_3, C–30), 17.9 (CH_3, C–18), 15.1 (CH_2, C–11); HRESIMS *m/z* 551.1571 ($[M+H]^+$, calcd for $C_{28}H_{33}O_7Cl_2$, 551.1603).

3.2.6 Conversion of compound **2** into compound **8**

Compound **2** (12 mg, 0.027 mmol) was dissolved in dichloromethane (2 mL), DMAP (1.7 mg, 0.014 mmol) and trichloroacetic anhydride {$(CCl_3CO)_2O$, 54 μL, 0.28 mmol} were added, and the resulting mixture was stirred for 72 h at room temperature. The solvent was removed under reduced pressure and the resulting residue was purified using ODS HPLC (MeOH/H_2O 65:35), resulting in compound **8** (8.1 mg, 51%).

Compound **8**: Colorless, amorphous solid; $[\alpha]_D^{27}$ + 20 (*c* 0.4, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40 (2H, s, H–21 and H–23), 7.09 (1H, d, *J*=10.2 Hz, H–1), 6.33 (1H, d, *J*=0.8 Hz, H–22), 5.86 (1H, d, *J*=10.2 Hz, H–2), 5.56 (1H, s, H–17), 4.68 (1H, br s, H–7), 3.58 (1H, s, H–15), 2.55 (1H, dd, *J*=12.2, 6.7 Hz, H–9), 2.27 (1H, d-like, *J*=13.4 Hz, H–5), 2.11 (1H, m, H–6), 2.00 (1H, m, H–11), 1.90 (1H, m, H–6), 1.87 (1H, m, H–11), 1.74 (1H, m, H–12), 1.61 (1H, m, H–12), 1.24 (3H, s, H_3–19), 1.23 (3H, s, H_3–18), 1.21 (3H, s, H_3–30), 1.09 (3H, s, H_3–28), 1.06 (3H, s, H_3–29); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 203.7 (C, C–3), 166.4 (C, C–16), 160.9 (C, C–1'), 156.8 (CH, C–1), 143.1 (CH, C–23), 141.3 (CH, C–21), 126.1 (CH, C–2), 120.2 (C, C–20), 109.9 (CH, C–22), 90.4 (C, C–2'), 78.8 (CH, C–7), 77.9 (CH, C–17), 69.3 (C, C–14), 57.8 (CH, C–15), 45.7 (CH, C–5), 44.1 (C, C–4), 43.0 (C, C–8), 40.2 (C, C–10), 39.2 (CH, C–9),

38.8 (C, C−13), 27.4 (CH_3, C−28), 25.6 (CH_2, C−12), 21.6 (CH_2, C−6), 21.3 (CH_3, C−29), 20.0 (CH_3, C−19), 18.4 (CH_3, C−30), 17.7 (CH_3, C−18), 15.0 (CH_2, C−11); HRESIMS *m/z* 585.1194 ($[M+H]^+$, calcd for $C_{28}H_{32}O_7Cl_3$, 585.1214).

Acknowledgements

The authors wish to sincerely thank Drs. Koichi Takeya, Yukio Hitotsuyanagi, Yutaka Aoyagi, and Tomoyo Hasuda of the Department of Natural Products and Medicinal Chemistry, Tokyo University of Pharmacy and Life Sciences for their careful guidance and encouragement throughout the conduct of this study.

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