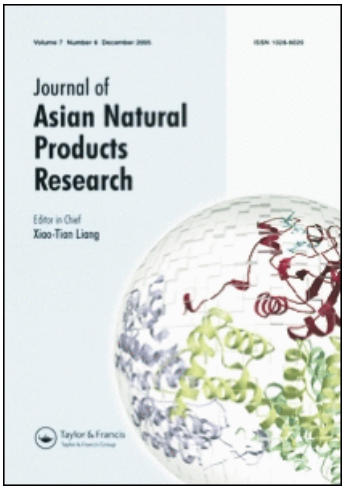

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ORIGINAL ARTICLE

Triterpenoids from the leaves of *Toona ciliata*

Jing Ning[ab], Hong-Ping He[a], Shi-Fei Li[ab], Zhao-Liang Geng[ab], Xin Fang[ab], Ying-Tong Di[a], Shun-Lin Li[a] and Xiao-Jiang Hao[a]*

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One new limonoid, toonaciliatone A (**1**), and one new tirucallane-type triterpenoid, toonaciliatine A (**4**), along with three known compounds, methyl-3β-acetoxy-1-oxomelic-14(15)-enate (**2**), perforin A (**3**), and cholest-14-ene-3,7,24,25-tetrol-21,23-epoxy-21-methoxy-4,4,8-trimethyl-3-(3-methyl-2-butenoate) (**5**), were isolated from the leaves of *Toona ciliata*. The structures of the new compounds were established by spectroscopic methods.

Keywords: *Toona ciliata*; limonoids; triterpenoid; tirucallane-type

1. Introduction

The plant of *Toona ciliata* Roem. var. *ciliata* (Meliaceae) is a rich source of structurally intriguing limonoids with diverse bioactivities [1–4]. Until now, a series of limonoids exhibiting different carbon frameworks and oxygenated patterns have been reported from this plant [5–8]. With the aim of searching for structurally unique and bioactive natural products, especially limonoids from the Meliaceae family, the chemical components of the leaves of *T. ciliata* have been investigated to give one new limonoid (**1**) and one new triterpenoid (**4**) (Figure 1). The isolation and structural elucidation of the new compounds and the cytotoxicity evaluation of all the isolated compounds are reported herein.

2. Results and discussion

Toonaciliatone A (**1**) was obtained as a white amorphous powder. The molecular formula was determined to be $C_{26}H_{34}O_5$ by the $[M + Na]^+$ ion peak at *m/z* 449.2303 in the HR-ESI-MS. The IR spectrum exhibited absorptions ascribable to hydroxyl (3437 cm^{-1}) and carbonyl (1724 cm^{-1}) groups. Its ^1H NMR spectrum exhibited the presence of five quaternary methyls at δ_H 0.81 (3H, s, Me-18), 1.04 (3H, s, Me-19), 1.11 (3H, s, Me-30), 1.34 (3H, s, Me-29), and 1.41 (3H, s, Me-28), two olefinic protons at δ_H 5.90 (1H, d, $J = 10.0$ Hz, H-2), and 7.05 (1H, d, $J = 10.0$ Hz, H-1), along with a β-substituted furan ring. The ^{13}C NMR spectrum (Table 1) of **1** further showed the presence of two ketonic carbonyls (δ_C 206.2 and 221.6) and two oxygenated carbons (δ_C 67.4 and 74.7). Careful investigation of the ^1H and ^{13}C NMR spectral data of **1** established that it was quite similar to those reported for 6α-acetoxy-14β,15β-epoxy-azadirone [5]. Comparison of the 1D NMR spectral data

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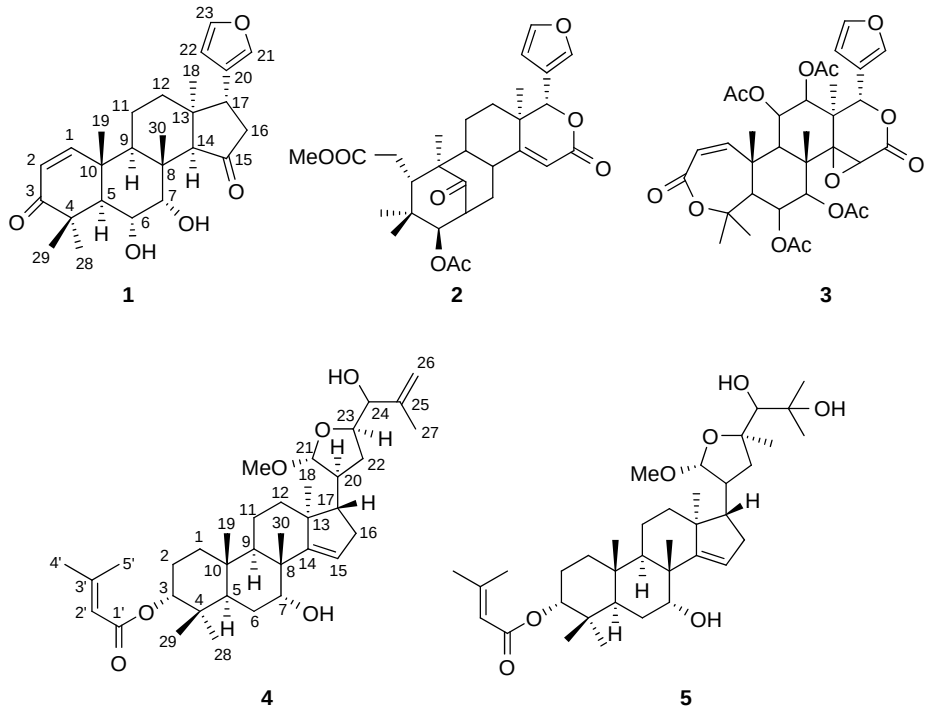

Figure 1. Structures of compounds **1**–**5**.

Table 1. ^{13}C NMR spectral data of **1** and **4** in $CDCl_3$ (δ in ppm, 100 MHz).

C	1	4	C	1	4
1	157.5 (d)	33.3 (t)	20	122.6 (s)	45.3 (d)
2	126.3 (d)	22.7 (t)	21	142.9 (d)	104.3 (d)
3	206.2 (s)	77.0 (d)	22	110.7 (d)	30.8 (t)
4	45.6 (s)	36.2 (s)	23	140.2 (d)	80.3 (d)
5	44.6 (d)	41.8 (d)	24		77.9 (d)
6	67.4 (d)	23.5 (t)	25		144.6 (s)
7	74.7 (d)	72.1 (d)	26		112.6 (t)
8	42.1 (s)	44.4 (s)	27		18.4 (q)
9	44.6 (d)	41.6 (d)	28	20.2 (q)	21.7 (q)
10	40.3 (s)	37.6 (s)	29	32.1 (q)	27.8 (q)
11	18.1 (t)	16.3 (t)	30	18.4 (q)	27.7 (q)
12	34.1 (t)	32.8 (t)	1′		166.5 (s)
13	42.1 (s)	46.6 (s)	2′		116.9 (d)
14	62.2 (d)	162.1 (s)	3′		155.7 (s)
15	221.6 (s)	119.6 (d)	4′		27.4 (q)
16	43.3 (t)	34.9 (t)	5′		20.2 (q)
17	38.1 (d)	52.5 (d)	OMe		54.7 (q)
18	27.7 (q)	19.8 (q)			
19	20.9 (q)	15.2 (q)			

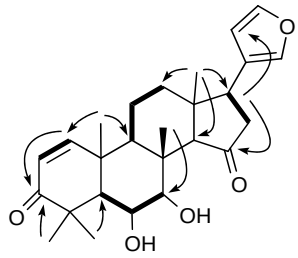

Figure 2. $^{1}H-^{1}H$ COSY (▬) and selected HMBC (→) correlations of **1**.

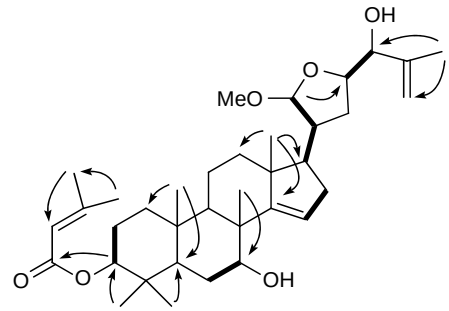

Figure 3. Selected $^{1}H-^{1}H$ COSY (▬) and HMBC (→) correlations of **4**.

with those of 6α-14β,15β-epoxy-azadirone, and the analysis of the HSQC, HMBC, and $^{1}H-^{1}H$ COSY data indicated that the two compounds had the same carbon backbone. The differences between them were that the 14β,15β-epoxy ring and 6,7-diacetyl groups in the latter were replaced by 15-ketone and 6,7-dihydroxy groups in compound **1**, respectively. This conclusion was also supported by the HMBC correlations of H-14 and H-16 with C-15 (δ_C 221.6, s) and the cross-peaks of OH-7/H-7 and H-7/H-6 in the $^{1}H-^{1}H$ COSY spectrum (Figure 2). In the ROESY experiment, correlations of H-14/OH-7 and Me-30/H-6 indicated that OH-6 and OH-7 were of α-orientation. Thus, the structure of **1** was completely elucidated.

Triterpenoid (**4**) was obtained as a colorless oil. Its molecular formula was determined as $C_{36}H_{56}O_6$ by HR-ESI-MS data at *m/z* 607.3969 $[M + Na]^+$. Its IR absorption bands at 3442 and 1656 cm^{-1} suggested the presence of hydroxyl and double bond functions, respectively. In the ^{1}H NMR spectrum, the signals of eight methyls and one β-substituted furan ring were observed, together with two olefinic protons at δ_H 5.46 (1H, d, $J = 9.5$ Hz, H-15) and 5.77 (1H, s, H-2′). Its ^{13}C NMR spectrum further showed the presence of four oxygenated methines (δ_C 77.0, 80.3, 77.9 and 104.3) and one ester carbonyl (δ_C 166.5). Comparison of the NMR spectral data of **4** with those of **5** found an overall similarity, except for the apparent different chemical shifts of C-25, C-26, and C-27 [9]. The difference between these two compounds was due to an occurrence of a double bond [δ_H 5.05 (1H, s) and 4.93 (1H, s); δ_C 144.6 (s) and 112.6 (t)] between C-25 and C-26 in **4**. Extensive 2D NMR experiments (HSQC, HMBC, $^{1}H-^{1}H$ COSY, and ROESY), especially the HMBC correlations of Me-27 with C-24, C-25, and C-26, confirmed the structure of **4** (Figure 3).

The three known compounds were identified as methyl-3β-acetoxy-1-oxomelic-14(15)-enate (**2**) [10], perforin A (**3**) [11], and cholest-14-ene-3,7,24,25-tetrol-21,23-epoxy-21-methoxy-4,4,8-trimethyl-3-(3-methyl-2-butenoate) (**5**) by comparison of their 1D NMR data with those in the literature [9].

Compounds **1**–**5** were tested for *in vitro* inhibitory activities against HL-60, SMMC-7721, A549, SK-BR-3, and PANC-1 human tumor cell lines (for more details, see Supporting Information). The results indicated that all the compounds were inactive against the above tumor cell lines (with $IC_{50} > 40$ μM).

3. Experimental

3.1 General experimental procedures

IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with a KBr disk. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. NMR spectra were measured on either a Bruker AM-400

or a DRX-500 instrument. ESI-MS and HR-ESI-MS spectra were measured with a Finnigan MAT 90 instrument and a VG Auto Spec-3000 spectrometer, respectively. Column chromatography was performed on silica gel (90–150 μm; Qingdao Marine Chemical Inc., Qingdao, China), MCI gel (CHP20P, 75–150 μm; Mitsubishi Chemical Industries Ltd, Japan), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and C18 reverse-phase silica gel (150–200 mesh; Merck, Darmstadt, Germany). Semi-preparative HPLC was performed on a Zorbax SB-C-18 column (i.d. 9.4 × 250 mm; Agilent Co. Ltd, Santa Clara, USA). TLC plates were pre-coated with silica gel GF-254 and HF-254 (Qingdao Haiyang Chemical Plant, Qingdao, China).

3.2 Plant material

The leaves of *T. ciliata* were collected in Wenshan, Yunnan Province, China, in July 2007, and were identified by Prof. Heng Li. A voucher specimen (No. 2007-5-10) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

3.3 Extraction and isolation

The air-dried and powdered leaves (2.6 kg) were extracted with EtOH (95%) three times. The extracts were then suspended in H_2O and further extracted with petroleum ether (PE), EtOAc, and *n*-BuOH, respectively. The EtOAc extracts (80 g) were subjected to silica gel CC eluting with PE–acetone (1:0, 9:1, 7:3, 0:1) to afford four fractions (A1–A4). Fraction A3 (9.2 g) was applied to MCI material (MeOH–H_2O, 70/30 to 100/0) to give four fractions, B1–B4. Fraction B3 (820 mg) was first subjected to Sephadex LH-20 (MeOH) and then to silica gel CC eluting with $CHCl_3$–acetone (100:1, 300 ml) and yielded **3** (24 mg). Fraction B2 (1.2 g) was subjected to Sephadex LH-20 (MeOH) to afford three fractions (C1–C3). Fraction C1 (56 mg) afforded **1** (20 mg) by semi-preparative HPLC (CH_3OH–H_2O, 60–40) (flow rate, 3.0 ml/min, detection, UV 210, 254 nm). Fraction B4 (1.6 g) was first subjected to reverse-phase C-18 silica gel (CH_3OH–H_2O, 70/30 (4 liters), 80/20 (2 liters), 90/10 (2 liters)) to afford three fractions (D1–D3). Fraction D2 (980 mg) was subjected to silica gel ($CHCl_3$–acetone: 300:1 (120 ml), 200:1 (100 ml), 80:1 (400 ml)) to afford compounds **2** (10 mg), **4** (10 mg), and **5** (50 mg), respectively.

3.3.1 Toonaciliatone A (*1*)

A white amorphous powder. $[\alpha]_D^{25}$ +22.2 ($c = 0.09$, $CHCl_3$). IR (KBr) ν_{max}: 3437, 1724, 1668, 756 cm^{-1}. ESI-MS *m/z*: 449 $[M + Na]^+$. HR-ESI-MS *m/z*: 449.2312 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_5Na$, 449.2303). ^1H NMR ($CDCl_3$, 400 MHz) δ_H: 0.81 (3H, s, Me-18), 1.04 (3H, s, Me-19), 1.11 (3H, s, Me-30), 1.34 (3H, s, Me-29), 1.41 (3H, s, Me-28), 1.31 (1H, dd, $J = 13.6$, 6.0 Hz, H_β-12), 1.38 (1H, dd, $J = 11.2$, 3.1 Hz, H-9), 2.04 (1H, dt, $J = 13.6$, 3.1 Hz, H_α-12), 1.66–1.72 (2H, m, H-11), 2.19 (1H, d, $J = 12.0$ Hz, H-5), 2.54 (2H, d, $J = 10.0$ Hz, H-16), 2.72 (1H, s, H-14), 3.15 (1H, d, $J = 4.4$ Hz, OH-7), 3.50 (1H, t, $J = 10.0$ Hz, H-17), 3.81 (1H, s, H-7), 4.16 (1H, t, $J = 12.0$ Hz, H-6), 5.90 (1H, d, $J = 10.0$ Hz, H-2), 7.05 (1H, d, $J = 10.0$ Hz, H-1), 6.30 (1H, s, H-22), 7.29 (1H, s, H-23), 7.41 (1H, s, H-21). ^{13}C NMR data ($CDCl_3$, 100 MHz): see Table 1.

3.3.2 Toonaciliatine A (*4*)

A colorless oil. $[\alpha]_D^{25}$ −151.7 ($c = 0.1$, $CHCl_3$). IR (KBr) ν_{max}: 3442, 3425, 1656, 1032 cm^{-1}. ESI-MS *m/z*: 585 $[M + H]^+$. HR-ESI-MS *m/z*: 607.3969 $[M + Na]^+$ (calcd for $C_{36}H_{56}O_6Na$, 607.3974). ^1H NMR ($CDCl_3$, 500 MHz) δ_H: 0.85 (3H, s, Me-28), 0.91 (3H, s, Me-29), 0.91 (3H, s,

Me-19), 1.03 (3H, s, Me-18), 1.06 (3H, s, Me-30), 1.77 (3H, s, Me-27), 1.89 (3H, s, Me-4′), 2.17 (3H, s, Me-5′), 1.26–1.32 (1H, m, H_α-1), 1.35–1.40 (1H, m, H_β-1), 1.50–1.54 (1H, m, H_β-12), 1.51–1.55 (1H, m, H_β-11), 1.61–1.65 (1H, m, H_α-12), 1.62–1.66 (1H, m, H_α-2), 1.70–1.74 (1H, m, H_α-11), 1.73–1.77 (1H, m, H_β-22), 1.74–1.78 (2H, m, H_2-16), 1.88–1.93 (1H, m, H_α-22), 1.99 (1H, d, $J = 3.1$ Hz, H-17), 2.02–2.06 (1H, overlapped, H-9), 2.02–2.06 (1H, overlapped, H-5), 2.14–2.20 (2H, m, H-16), 2.22–2.27 (1H, m, H-20), 3.36 (3H, s, 21-OMe), 3.83 (1H, d, $J = 4.5$ Hz, H-24), 3.91 (1H, s, H-7), 4.20–4.25 (1H, m, H-23), 4.69 (1H, s, H-3), 4.76 (1H, d, $J = 4.1$ Hz, H-21), 4.93 (1H, s, H_a-26), 5.05 (1H, s, H_b-26), 5.46 (1H, d, $J = 9.5$ Hz, H-15), 5.77 (1H, s, H-2′). ^{13}C NMR data: see Table 1.

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