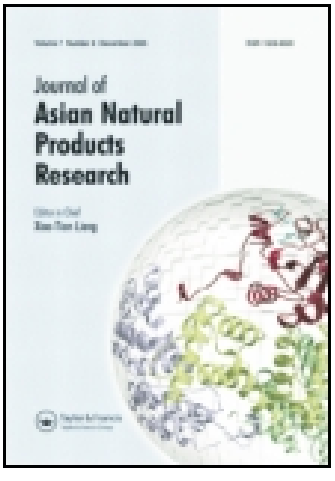

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Limonoids from the fruits of *Cipadessa cinerascens*

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A new limonoid, 3-de(2-methylbutanoyl)-3-propanoylcipadesin (**1**), along with 10 known limonoids and 1 known triterpenoid, was isolated from the fruits of *Cipadessa cinerascens*. Their structures were elucidated on the basis of spectroscopic analysis. All compounds were evaluated for their antimicrobial activities, and compounds **6** and **12** showed weak antimicrobial activities against MRSA 82# and MRSA 92#.

Keywords: *Cipadessa cinerascens*; limonoids; antimicrobial activities

1. Introduction

The genus *Cipadessa* (Meliaceae) comprises nine species of shrubs or small trees, which are mainly distributed in India and southwest of China [1]. The folk medicine "Ya Luo Qing", derived from the leaves and roots of *Cipadessa* plants, has been used for the treatment of dysentery, skin itch, malaria, and burns, by a Chinese minority-ethnic Dai [2]. Previous investigations of the genus have led to the isolation of a rich source of limonoids with diverse skeletons [3–7]. *Cipadessa cinerascens* is widely distributed in the southwest of Mainland China [8]. In continuation of our studies on the Meliaceae family [9–13], this paper reports the isolation of a new limonoid, 3-de(2-methylbutanoyl)-3-propanoylcipadesin (**1**), along with 10 known limonoids and 1 known tritepenoid, febrifugin (**2**) [14], khayasin T (**3**) [15], 2′*S*-cipadesin (**4**) [4], methyl-8α,30α-epoxide-3β-(2′-methylbutyryloxy)-1-oxomeliacate (**5**) [16], xylocarpin (**6**) [17], swietemahonolide (**7**) [18], swietemahonin F (**8**) [18], cipadesin D (**9**) [19], cipadesin H (**10**) [20], cipadesin I (**11**) [20], and mesendanin T (**12**) [21], from the fruits of the titled plant. All compounds were screened for their antimicrobial activities (Figure 1).

2. Results and discussion

3-De(2-methylbutanoyl)-3-propanoylcipadesin (**1**) had molecular formula $C_{30}H_{38}O_8$ on the basis of its molecular-ion peak $[M]^+$ at *m/z* 526.2556 in its HR-EI-MS, with 12 degrees of unsaturation. The ^{13}C NMR spectrum, along with DEPT experiments (Table 1), displayed 30 carbon resonances assignable to 6 methyls (1 methoxy), 5 methylenes, 10 methines (4 olefinic and 2 oxygenated), and 9 quaternary carbons (2 olefinic and 4 carbonyls). Apart from eight degrees of unsaturation consumed by a characteristic β-furan ring, four carbonyl groups, and a

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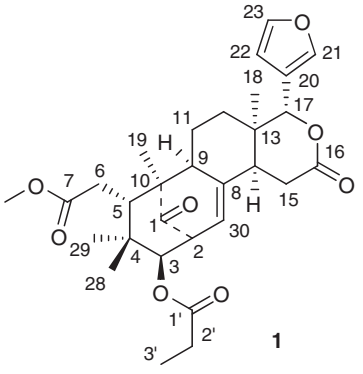

Figure 1. Structure of compound **1**.

double bond, the remaining four degrees of unsaturation suggested **1** to be tetracyclic. The aforementioned data suggested that **1** is a mexicanolide-type limonoid [4]. Further analysis of the 1D and 2D NMR spectra (Figure 2) indicated that **1** showed many similarities to those of 2′*S*-cipadesin (**4**) [4], except for the absence of a 2-methylbutanoyl group and the presence of a propanoyl group. In the HMBC spectrum, correlations from H-3 (δ_H 4.76, d, J = 9.3 Hz), Me-3′ (δ_H 1.12, t, J = 7.7 Hz), and H_2-2′ (δ_H 2.39–2.41, 2H, m) to the carbonyl of the propanoyl group (δ_C 174.2) revealed that the propanoyl group was attached to C-3. Thus, the gross structure of **1** was established as depicted.

The relative stereochemistry of **1** was assigned from the ROESY spectrum (Figure 2). The cross-peaks from H-5 to Me-28, H-17, and H-11β, indicated that those groups were cofacial, and these were arbitrarily assigned as β-orientation. In consequence, the ROESY correlations of Me-18/H-14, H-14/H-30, H-30/H-2, and H-2/H-3 revealed that they were α-oriented. Thus, the propanoyl group at C-3 was β-oriented. Accordingly, the structure of **1** was established as shown.

All compounds (**1**–**12**) were screened for their antimicrobial activities against four microorganisms, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, MRSA (methicillin-resistant *S. aureus*) 92#, and MRSA 98#. The minimum inhibitory concentrations (MICs) of **5**, **6**, and **12** were determined by the twofold dilution method [22,23]. The results revealed that two compounds (**6** and **12**) showed weak antimicrobial activities against MRSA 82# and MRSA 92# with an MIC value of 50 μg/ml (Table 2).

Table 1. ^{1}H NMR (500 Hz) and ^{13}C NMR (125 Hz) spectroscopic data for compound **1** in $CDCl_3$.

Position	δ_H (J, Hz)	δ_C	Position	δ_H (J, Hz)	δ_C
1		217.3	15β	2.79–2.81 (1H, m)	
2	3.50–3.52 (1H, m)	48.5	16		169.7
3	4.76 (1H, d, 9.3)	77.3	17	5.69 (1H, s)	77.2
4		38.3	18	1.08 (3H, s)	21.9
5	3.37 (1H, dd, 7.4, 4.0)	41.5	19	1.14 (3H, s)	15.8
6	2.36–2.38 (2H, m)	32.9	20		120.6
7		174.1	21	7.79 (1H, s)	141.9
8		138.4	22	6.46 (1H, s)	109.7
9	2.21–2.23 (1H, m)	56.5	23	7.41 (1H, s)	142.9
10		49.9	28	0.81 (3H, s)	20.3
11α	1.65–1.67 (1H, m)	20.4	29	0.78 (3H, s)	22.4
11β	2.10–2.12 (1H, m)		30	5.34 (1H, d, 7.2)	122.8
12α	1.39–1.41 (1H, m)	34.3	7-OCH_3	3.71 (3H, s)	52.2
12β	1.64–1.66 (1H, m)		1′		174.2
13		36.7	2′	2.39–2.41 (2H, m)	27.1
14	2.23–2.25 (1H, m)	45.1	3′	1.12 (3H, t, 7.7)	8.8
15α	2.87–2.89 (1H, m)	29.9			

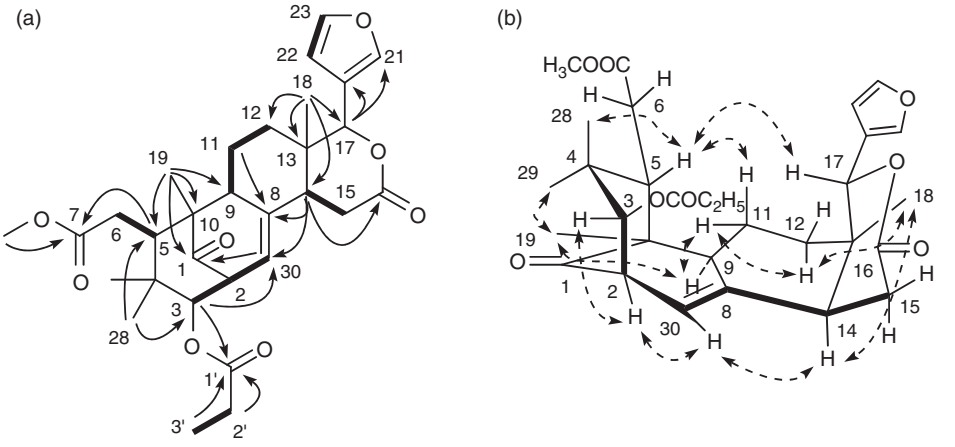

Figure 2. Key HMBC (a), $^{1}H-^{1}H$ COSY (a), and ROESY (b) correlations of compound **1**.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a JASCO P-1020 digital polarimeter (Jasco, Tokyo, Japan). UV spectra were detected on a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra were scanned with Bruker Tensor-27 infrared spectrometer with a KBr disk (Bruker, Karlsruhe, Germany). Brucker HCT/E squire (Bruker) and Waters Autospec Premier P776 spectrometers (Waters, Millford, MA, USA) were used to measure ESI-MS and HR-EI-MS, respectively. 1D and 2D NMR spectra were recorded on a Bruker AM-400 and DRX-500 spectrometers (Bruker) with trimethylsilyl as internal standard. Column chromatography was performed on silica gel (200–300 and 300–400 mesh; Qingdao Marine Chemical, Inc., Qingdao, China), MCI gel CHP 20P (75–150 mm; Mitsubishi Chemical Corporation, Tokyo, Japan), Sephadex LH-20 (40–70 mm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Chromatorex Rp-C_{18} gel (20–45 mm; Merck, Darmstadt, Germany). Thin layer chromatography (TLC plates; Qingdao Marine Chemical, Inc.) spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH followed by heating.

3.2 Plant material

The dried fruits of *C. cinerascens* were collected in Chuxiong, Yunnan Province of China in November 2011, and were identified by Mr Yu Chen (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (No. H20111102) has been deposited at the Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming

Table 2. Antimicrobial activities of **6** and **12**.

	MICs (μg/ml)	
Compounds	MRSA 82#[a]	MRSA 92#[a]
6	50	50
12	50	50
Vancomycin hydrochloride[b]	0.78	0.78

[a] MRSA (methicillin-resistant *S. aureus*) 82# and MRSA (methicillin-resistant *S. aureus*) 98#.
[b] Positive control.

Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried powdered fruits (28.0 kg) were extracted with MeOH. The combined MeOH extracts were concentrated under vacuum to give a crude residue, which was suspended in water and then partitioned successively with $CHCl_3$. The $CHCl_3$ portion (3.2 kg) was chromatographed on a silica gel column, eluted with petroleum ether–ethyl acetate (from 10:0 to 4:6) to yield 10 fractions (Fr. 1–10). Fr. 3 (398.1 g) was then separated on a silica gel column (petroleum ether–acetone from 9:1 to 0:10) to obtain eight fractions (3A–3H). Fr. 3C (3.0 g) was chromatographed on Sephadex LH-20 (MeOH) to give **3** (161.9 mg), **4** (11.9 mg), and a subfraction 3C2 (400 mg), which was further purified by a silica gel column ($CHCl_3$–Me_2CO, 100:1) to obtain **2** (10.9 mg), **7** (46.4 mg), and **8** (25.1 mg). Fr. 3D (50.0 g) was then separated over an MCI-gel column (MeOH–H_2O from 2:8 to 10:0) to obtain four fractions (Fr. 3D1–3D4). Fr. 3D1 (800 mg) was purified by Sephadex LH-20 (MeOH), and then chromatographed on a silica gel column ($CHCl_3$–Me_2CO, 100:2) to obtain **1** (5.6 mg), **5** (10.0 mg), and **12** (10.9 mg). Fr. 3D3 (1.61 g) was chromatographed on a C_{18} silica gel column, eluted with a gradient MeOH–H_2O (from 40:60 to 80:20) to afford five subfractions (3D3A–3D3E). Fr. 3D3C (400 mg) was then purified by HPLC (MeOH–H_2O, 65:35) to yield **6** (17.4 mg), **9** (6.6 mg), **10** (8.4 mg), and **11** (11.5 mg).

3.3.1 3-De(2-methylbutanoyl)-3-propanoyl-cipadesin (1)

White amorphous power; $[\alpha]_D^{16}$ −113.6 (c = 0.18, CH_3OH); UV (MeOH) λ_{max} (log ε) nm 206 (4.0); IR (KBr) ν_{max} 2926, 2855, 1729, 1630, 1383, 1221, 1172, 1027 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; positive ESI-MS: m/z 549 $[M + Na]^+$; HR-EI-MS: m/z 526.2556 $[M]^+$ (calcd for $C_{30}H_{38}O_8$, 526.2567).

3.4 Antimicrobial assays

The strains used in antimicrobial tests were obtained from the Research Center of Natural Medicine, Clinical School of Kunming General Hospital of Chengdu Military Command. For the agar plate punch assay [22], all compounds were dissolved in DMSO at a concentration of 1000 μg/ml. Then, 50 μl of the solution was added onto a well (6 mm in diameter) that had been punched in the appropriate agar growth medium smeared with a suspension of the test organism (1.5×10^9 cfu/ml; cfu, colony forming unit). The test organisms in this bioassay were the bacteria, *S. aureus*, *P. aeruginosa*, MRSA 92#, and MRSA 98# (clinically isolated strains, from Kunming General Hospital of Chengdu Military Command), and all were grown on MH medium. Compounds **5**, **6**, and **12** with a diameter of inhibition greater than 10 mm against MRSA 92# were subjected to MIC testing. The MICs of compounds **5**, **6**, and **12** against MRSA 82# and MRSA 92# were determined using a twofold dilution method [22]. The twofold serially diluted compounds in MH broth were dispensed into 96-well microtiter plates (100 μl/well), and then an aliquot of 5×10^5 cfu/ml of bacterial culture was added to each well (100 μl/well) to a final concentrations in the range of 0.78–50 μg/ml. After incubating at 37°C for 18 h, the lowest concentration without any colony growth was recorded as the MIC value. The resulting values were compared with the value for a positive control (vancomycin hydrochloride, the MIC value: 0.78 μg/ml) under the same conditions.

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