SHORT REPORT

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Antibacterial triterpenoids from *Melia toosendan*

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Abstract: A new lanostan-type triterpenoid with hydroperoxy group, toosendanin A (**1**), together with two known triterpenoids, meliastatin 3 (**2**) and ursolic acid (**3**), were isolated and identified from the stems of *Melia toosendan*. The structures of these compounds were elucidated by 1D- and 2D-NMR spectra and other spectroscopic studies. These compounds were assayed for the antibacterial activities against some hospital pathogenic bacteria. Toosendanin A (**1**) exhibited strong antibacterial activity against *K. pneumoniae*.

Keywords: *Melia toosendan*; triterpenoids; Meliaceae; antibacterial.

1. Plant Source

In this paper, we present the chemical investigation of the stems of *Melia toosendan* Sieb. et Zucc (Meliaceae) from West of China. A novel lanostan-type triterpenoid with hydroperoxy group, namely toosendanin A (**1**), was isolated and identified (Figure 1).

The stems of *M. toosendan* were collected in Sichuan province of West China in April 2004, and identified by Prof. Jingui Shen. A voucher (SIMM-YYE-200405) was deposited at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2. Previous Studies

Melia toosendan Sieb. et Zucc. (Meliaceae), the Chinaberry tree, has long been recognized as an insecticidal and medicinal plant in China [1]. Its fruits, with the name 'Chuan-Lian-Zi' in Chinese, are used for treatment of malaria, for stomach aches caused by roundworms, and even as an insecticide [2]. Former chemical and pharmacological studies have found limonoids are responsible for its bioactivities. A number of limonoids have been isolated from the fruits and the most active constituents are the azadirachtin-type C-seco-limonoids [3]. Intact apo-euphol limonoids, such as the meliasanins [4] with a 14,15-epoxide and a C-19/C-29 lactol bridge are also active. Lanostan-type triterpenoids are reported from this specie [5,6].

3. Present Study

20 kg air-dried stems of *M. toosendan* were ground into powder and extracted with 95% ethanol (60 L×3). After evaporation of the collected percolate, the crude extract (580 g) was

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partitioned with CH_2Cl_2, EtOAc, and *n*-BuOH, respectively. The EtOAc fraction (36 g) was subjected to column chromatography over silica gel and eluted gradient with chloroform-MeOH from 99:1 to 10:1, and methanol. 12 fractions were successively obtained. Fraction 6 (980 mg) was then separated over a Sephadex LH-20 gel column ($CHCl_3$-MeOH = 1:1). Successive isolation of subfraction 2 (387 mg) over RP-18 silica gel, eluting with MeOH-H_2O from 5:1 to 1:0, gave **1** (58 mg) and **2** (124 mg). Subfraction 3 (36 mg) was separated over silica gel and eluted with cyclohexane-EtOAc from 3:1 to 1:1, to obtain **3** (9 mg).

*Toosendanin A **(1)**:* White amorphous powder (MeOH-H_2O, 5:1); $[\alpha]_D^{20} = -27$ (c = 0.217, $CHCl_3$); IR ν_{max} (KBr): = 3427, 2949, 1709, 1437, 1387, 1171 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): see Table 1; EIMS (rel. int.): *m/z* 516 $[M]^+$ (1), 483 (12), 466 (31), 451 (64), 433 (60), 397 (94), 381 (28), 337 (24), 295 (100), 272 (26); ESIMS: *m/z* 539.4 $[M+Na]^+$, 561.6 $[M+HCOOH]^-$; HRMSESI: *m/z* [M + Na^+] calcd for $C_{31}H_{48}O_6Na$: 539.3349; found: 539.3358.

Antibacterial assay: The anti-bacterial activity was determined using broth dilution techniques as previously described in the literature [7]. The solutions (maximum concentration) of the compounds (i.e. the compounds that induced zones of inhibition) were prepared in DMSO, serially (2-fold) diluted and 0.5 mL of each dilution was introduced into a test tube containing 4.4 mL of Selenite broth; then 0.1 mL of bacteria suspension (5×10^5 cfu/mL) was added and the mixture was homogenized. The total volume of the mixture was 5 mL, with the test-compound concentrations in the tube ranging from 400 to 12.5 μg/mL and those of the standard compounds, i.e. Gentamycin and Amphotericin B, ranging from 50 to 1.562 μg/mL and 50 to 1.562 μg/mL, respectively. After 24 h of incubation at 37 °C, the MIC was reported as the lowest concentration of anti-microbial that prevented visible growth.

A 95% EtOH extract of the stems of *M. toosendan* (20 kg) was suspended in H_2O and successively extracted with CH_2Cl_2, EtOAc, and *n*-BuOH. The EtOAc fraction was subjected to column chromatography to yield three triterpenoids, toosendanin A (**1**), meliastatin 3 (**2**) and ursolic acid (**3**) (Figure 1).

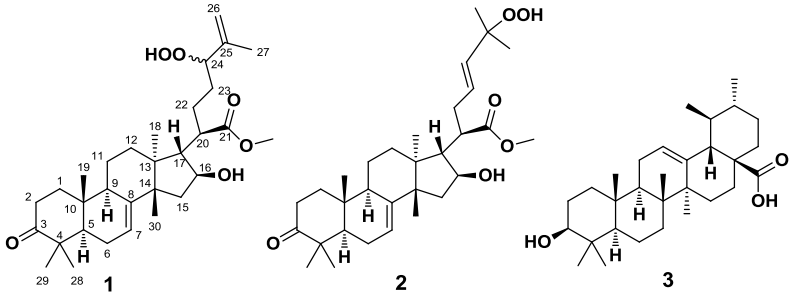

Figure 1. Triterpenoids isolated from *M. toosendan.*

Toosentanin A (**1**) was obtained as a white amorphous powder. Its EI-MS gave the molecular ion peak at *m/z* 516 $[M]^+$. Its molecular formula was deduced to be $C_{31}H_{48}O_6$ on the basis of ion peak at *m/z* 539.3358 (calcd. for 539.3349 $C_{31}H_{48}O_6Na$) in HR-ESI-MS, with eight degrees of unsaturation. Its IR spectrum displayed a broad and strong peak at 3427 cm^{-1} characteristic for hydroxyl group, and a sharp peak at 1709 cm^{-1} for the existence of ester carbonyl group. The ^1H NMR spectrum of **1** (Table 1) showed six singlet methyl, a methoxyl at δ_H 3.72, three olefinic protons at δ_H 5.30 (1H, m), 5.05 (1H, s) and 5.02 (1H, s), and two protons bearing an oxygen functionality at δ_H 4.30 (1H, m) and 4.00 (1H, m). The ^{13}C NMR (Table 1) and DEPT spectra of **1** revealed the presence of seven tertiary methyls, eight sp^3-hybridized methylenes, six sp^3-methines including two oxygen-bearing methines, four quaternary sp^3-carbons, 1,1-di- and trisubstituted double bonds, one ester and one ketone. Considering the unsaturation, compound **1** was suggested to be a tetracyclic triterpenoid. The ^1H-^1H COSY analysis of **1** led to four partial structural units, which were supported by HMBC correlations (bold lines in Figure 2a). The connections of these units were determined on the basis of key HMBC correlations (arrows in Figure 2a), and the basic skeleton of **1** was constructed, which was the same as meliastatin 4 [8]. Compound **1** contained one oxygen atom more than meliastatin 4 by compared their molecular formula. The ^1H and ^{13}C NMR spectra of **1** resembles to those of meliastatin 4, except the downfield shift of H-24 from 4.03 to 4.30 and C-24 from 75.8 to 89.1. It let us suppose that a

hydroperoxy group annexed at C-24 in **1** displaced the hydroxyl group at the same place of meliastatin 4. The key HMBC correlations between H-C(26) (δ 5.02 (*s*) and 5.05 (*s*)), Me-(27) (δ 1.72 (*s*)) and C-24 (δ 89.1) further supported the existence of a hydroperoxy group at C-24 (arrows in Figure 2a). Thus the planar structure of **1** was thereby elucidated and the signal assignments in detail were shown in Table 1.

Table 1. The ^{1}H-NMR and ^{13}C-NMR data of compound **1** (δ ppm, *J* Hz) (in $CDCl_3$)

No.	δ_H (*J*)	δ_C	No.	δ_H (*J*)	δ_C
1	1.49 (m), 1.91 (m)	38.4	17	2.00 (m)	58.5
2	2.24 (m), 2.74 (m)	34.9	18	0.83 (s)	23.5
3		216.8	19	1.05 (s)	12.7
4		47.9	20	2.58 (m)	47.3
5	1.61 (m)	52.3	21		177.3
6	1.37 (m), 2.16(m)	24.3	22	1.59 (m), 1.62 (m)	17.9
7	5.30 (m)	118.6	23	1.27 (m), 1.42 (m)	28.2
8		144.5	24	4.30 (m)	89.1
9	2.28 (m)	47.9	25		143.1
10		35.0	26	5.02 (s), 5.05 (s)	114.8
11	0.98 (m), 1.00 (m)	27.2	27	1.72 (s)	17.1
12	1.59 (m), 1.90 (m)	32.9	28	1.05 (s)	24.5
13		45.4	29	1.12 (s)	21.5
14		49.7	30	1.29 (s)	27.8
15	1.58 (m), 1.97 (m)	44.6	OMe	3.72 (s)	51.9
16	4.00 (m)	77.2			

The stereochemistry of **1** was determined with ROESY experiment (broken arrows in Figure 2b). The NOE correlations from H-19 to H-29 and H-2β and from H-1α to H-5 indicated that the A-ring exists in a chair conformation with H-5 and H-19 in a *trans*-diaxial arrangement. And ring A and ring B were *trans* fusion. In addition, NOEs from H-5 to H-9 and H-6α and from H-29 to H-6β implied that the ring B exists in a twist chair conformation with H-9 in an axial arrangement. Furthermore, the NOEs from H-30 to H-11β, H-12β, and H-19 and from H-18 to H-9 indicated that the C-ring exists in a twist boat conformation with H-18 and H-30 in a *trans*-diaxial arrangement, suggesting the *trans* fusion of ring C to ring D. NOE correlations between H-18 and H-16 and between H-30 and H-17 showed that H-16 and H-17 are oriented *trans*-diaxially. The observation of NOEs from H-20 to H-18 and H-16 indicated the relative configuration of C-20 to be *rel-R*. The above evidence established the complete structure of **1**.

All these compounds were assayed for the antibacterial activities against three hospital pathogenic bacteria, *B. pumilus* (ATCC 21356), *C. neoformans* (ATCC 34543) and *K. pneumoniae* (ATCC BAA-1705) *in vitro*. Two antibacterial agents, Gentamycin and Amphotericin B, were used as positive controls in these tests. Among the tested compounds, toosendanin (**1**) showed strong antimicrobial activities against *K. pneumoniae* at the level of MIC 100 μg/mL (Table 2). Meanwhile, compounds **2** and **3** also showed strong antibacterial activities against *K. pneumoniae*. All three compounds had no inhibitory activities on the other two bacteria.

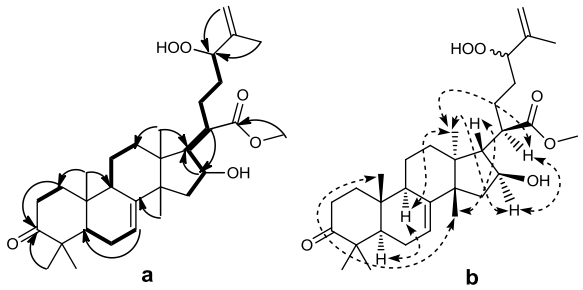

Figure 2. **a**. Important ^{1}H-^{1}H COSY (bold lines) and HMBC correlations (arrows) of **1**. **b**. Key ROESY correlations (broken arrows) in **1**.

Table 2. Antibacterial activities of compounds **1**–**3**

Compounds and controls	Minimum inhibitory concentration (μg/mL)		
	B. pumilus	*C. neoformans*	*K. pneumoniae*
1	200	200	100
2	200	>200	>50
3	200	200	100
Gentamycin	3.125	>200	3.125
Amphotericin B	>200	1.562	>200

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP.

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