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THREE NEW LIMONOIDS FROM THE FRUITS OF *MELIA TOOSENDAN*

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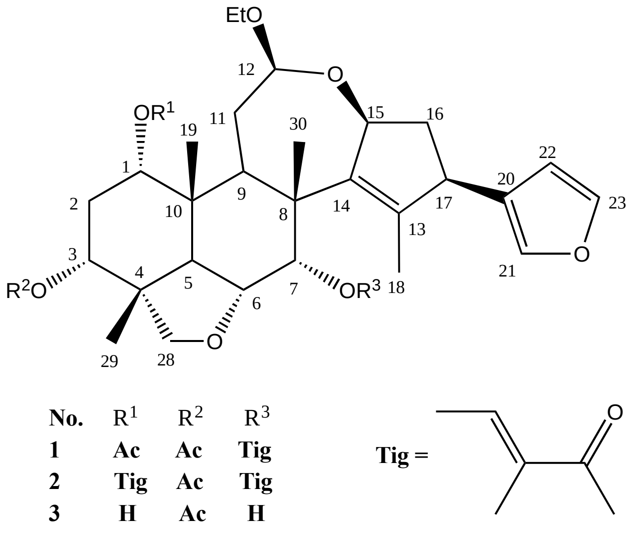
Abstract – Three new limonoids (**1-3**) were isolated from the fruits of *Melia toosendan*. Their structures were established on the basis of spectroscopic methods. In addition, compounds **1-3** were evaluated for their antibacterial activities.

Melia toosendan Sieb. et Zucc. (Meliaceae) is a wild plant growing mainly in the southwestern part of China. The fruits of *M. toosendan* have been used for treatment of acesodyne and desinsection in traditional Chinese medicine.[1] Its chemical constituents reported in previous investigations included highly functionalized limonoids such as meliacins, trichilinins, C-19/C-29-bridged acetals, ring C-seco

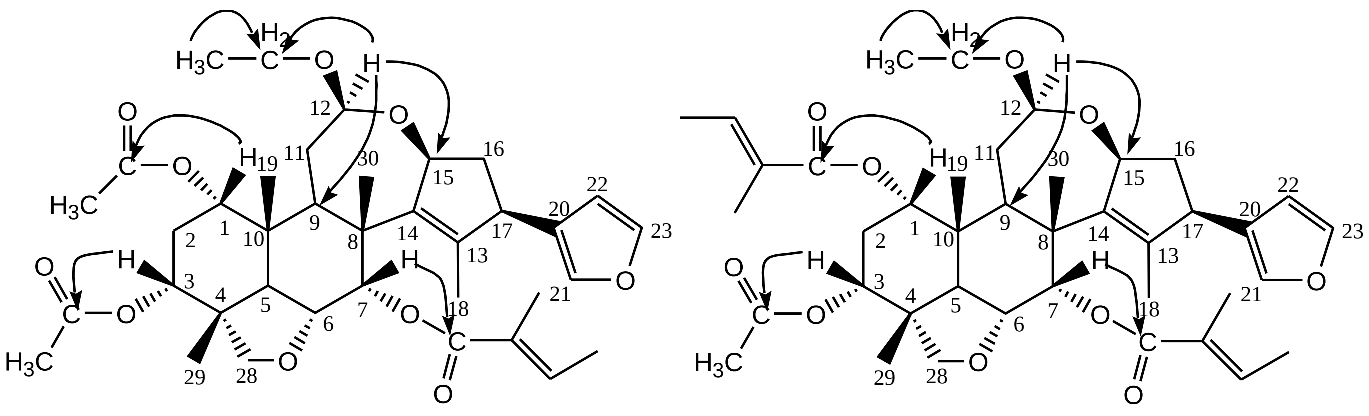


No.	R1	R2	R3
1	**Ac**	**Ac**	**Tig**
2	**Tig**	**Ac**	**Tig**
3	**H**	**Ac**	**H**

Figure 1. Structures of compounds **1-3**

limonoids, highly oxidized C-seco limonoids, spiro limonoids, and euphane- and tirucallane-type triterpenoids.[2-5] Limonoids, the major metabolites of *M. toosendan*, possess various biological activities,such as antifeedant, antimicrobial, antiprotozoal, anti-inflammatory, and anticancer activities.[2-10] Limonoids from *Melia* species are attracting considerable interest because of their biological activities and variety of structures. In our continued studies on the bioactive limonoids from *M. toosendan*, three new limonoids (**1**-**3**) (Figure 1) were isolated. The antibacterial activity of the isolated compounds against two oral pathogens (*Streptococcus mutans* ATCC 25175 and *Porphyromonas gingivalis* ATCC 33277) had been determined. Herein, we describe about the isolation, structure elucidation, and the biological evaluation of these compounds.

Compound **1**, amorphous powder, was assigned the molecular formula $C_{37}H_{50}O_{10}$ based on its HR-ESI-MS. The IR spectrum showed absorption peaks at 3417 cm^{-1} (hydroxy group) and 1737 cm^{-1} (carbonyl group). The ^{1}H-NMR and ^{13}C-NMR data of **1** (Table 1) demonstrated that compound **1** was consisted of a tigloyl group [δ_H 6.92 (1H), 1.77 (3H), 1.90 (3H), δ_C 166.4 (CO), 136.6, 128.8, 14.3, 12.1], two acetyl groups [δ_H 1.99 (δ_C 20.8), δ_C 169.9] [δ_H 2.07 (δ_C 21.6), δ_C 169.7], and an ethoxy group [δ_H 1.13 (3H, t, J=7.1Hz), δ_C 15.5, δ_H 3.54 (1H, m), 3.13 (1H, m), δ_C 63.6]. A furan ring [δ_H 6.32 (1H), 7.21 (1H), 7.27 (1H), δ_C 128.5, 110.2, 138.8, 142.9] was also apparent from the NMR spectra. By comparison the remaining NMR data with those limonoids isolated from *M. toosendan* before, a ring C-seco nimbolinin skeleton was assigned. The presence of the tigloyl group at the C-7 position was confirmed by the observation of the $^{3}J_{CH}$ connectivities in HMBC spectrum (Figure 2) between H-7 (δ_H 5.79) and the carbonyl of the tigloyl group (δ_C 166.4). In addition, according to the HMBC spectrum, the two acetyl groups were placed at C-1 and C-3 respectively and the ethoxy group was situated at C-12. The planar structure of compound **1** was the same as 12-*O*-ethylnimbolinin B.[9,11] The stereochemistry of **1** was established by a NOESY experiment (Figure 3). The NOE correlations of Me-29/H-3, Me-29/H-6 and Me-29/Me-19 suggested the β-orientation of H-3 and thus the β-orientations of Me-29, H-6 and Me-19. The NOE correlation of Me-19/H-1 revealed that H-1 was in the β-configuration. The NOE correlations between H-7/H-6 and H-7/Me-30 suggested that H-7 was in the β-configuration, while the NOE correlations of H-15/H-16α and H-17/H-16α implicated α-configurations for H-15 and H-17. Observation of NOE effects between H-9/H-5, H-9/H-12, and H-9/H-15 indicated the α-configuration of H-12, which deduced a significant downfield shift for C-12 (δ_C 103.9). Thus, the structure of compound **1** was elucidated as 1α,3α-diacetoxy-7α-tigloyloxy-12β-ethoxynimbolinin.



1 2

Figure 2. Key HMBC correlations of compounds **1** and **2**

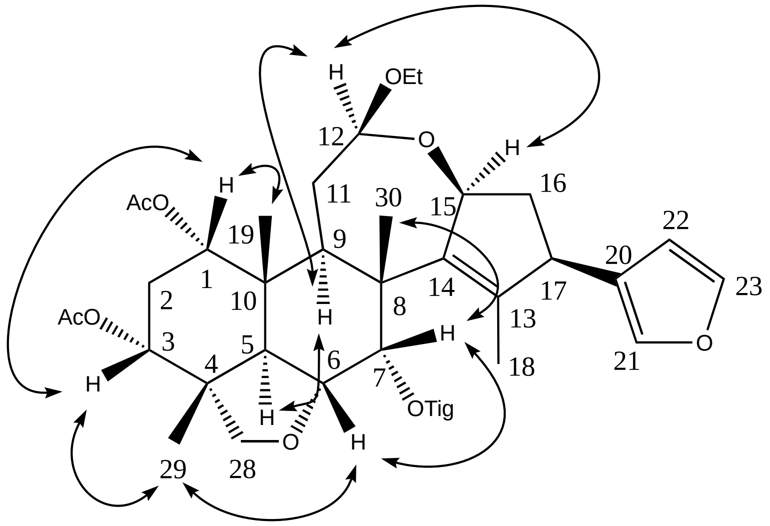


Figure 3. Key NOESY (HH) correlations of compound **1**

Compound **2**, isolated as an amorphous powder, had a molecular formula of $C_{40}H_{54}O_{10}$ deduced from HR-ESI-MS. The ^{1}H-NMR and ^{13}C-NMR spectra (Table 1) showed the presence of an acetyl group [(δ_H 1.92 (δ_C 20.8), δ_C 169.9) and two tigloyl groups [δ_H 6.83 (1H), 1.79 (3H), 1.88 (3H), δ_C 166.9 (CO), 136.2, 128.7, 14.3, 12.3] [δ_H 6.95 (1H), 1.78 (3H), 1.96 (3H), δ_C 166.8 (CO), 136.5, 129.5, 14.2, 12.4]. On the basis of the NMR data, the same carbon skeleton as compound **1** was proposed. According to the HMBC spectrum, the two tigloyl groups were placed at C-1 and C-7 respectively, and the acetyl group was linked to C-3 (Figure 2). The planar structure of compound **2** was the same as 1α,7α-ditigloyloxy-3α-acetoxy-12α-ethoxynimbolinin.[9] The NOESY experiment showed that the stereochemistry of compound **2** was consistent with compound **1**. Therefore, the structure of compound **2** was established as 1α,7α-ditigloyloxy-3α-acetoxy-12β-ethoxynimbolinin.

Compound **3** was obtained as an amorphous powder. In the HR-ESIMS, it gave a quasi-molecular ion peak at *m/z* 553.2876 [M+Na]$^+$, corresponding to a molecular formula $C_{30}H_{42}O_8$. The IR spectrum showed absorption bands at 3445 and 1719 cm^{-1}, which were in accordance with hydroxy and carbonyl groups. An acetyl group [δ_H 2.12 (δ_C 21.6), δ_C 171.5] and an ethoxy group [δ_H 1.23 (3H, t, *J*=7.1 Hz), δ_C 15.1, δ_H 3.76 (1H, m), 3.53 (1H, m), δ_C 64.0] were obviously observed in the NMR spectra. On the basis of the NMR data, the same carbon skeleton as compounds **1** and **2** was proposed. According to the HMBC spectrum, the acetyl group was situated at C-3 and the two hydroxy groups were located at C-1 and C-7 respectively. The relative configuration of **3** was determined by a NOESY spectrum and it was consistent with compounds **1** and **2**. Therefore, the structure of compound **3** was characterized as 1α,7α-dihydroxy-3α-acetoxy-12β-ethoxynimbolinin.

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1-3** (500 MHz for ^{1}H and 125 MHz for ^{13}C, in $CDCl_3$, δ in ppm, *J* in Hz)

position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.85 (1H, m)	71.7	4.96 (1H, m)	71.7	3.72 (1H, m)	71.4
2a	2.19 (1H, m)	27.8	2.20 (1H, m)	27.9	2.13 (2H, m)	29.3
2b	2.14 (1H, m)		2.05 (1H, m)			
3	4.99 (1H, m)	71.3	4.99 (1H, m)	71.2	4.96 (1H, m)	71.8
4		42.3		42.6		42.7
5	2.78 (1H, d, 12.8)	40.0	2.75 (1H, d, 12.8)	40.8	2.90 (1H, m)	37.5
6	4.05 (1H, dd, 12.8, 2.9)	72.3	4.12 (1H, dd, 12.8, 3.0)	72.4	4.05 (1H, dd, 12.7, 2.9)	74.6
7	5.79 (1H, d, 2.9)	74.7	5.84 (1H, d, 3.0)	74.4	4.28 (1H, d, 2.9)	73.9
8		45.4		45.5		45.9
9	2.63 (1H, m)	38.4	2.57 (1H,m)	39.3	2.49 (1H, m)	37.5
10		40.4		40.6		41.2
11a	1.78 (1H, m)	31.9	1.78 (1H, m)	31.8	1.76 (1H, m)	30.9
11b	1.53 (1H, m)		1.55 (1H, m)		1.55 (1H, m)	
12	4.01 (1H, br s)	103.9	3.98 (1H, br s)	103.7	4.15 (1H, br s)	103.9
13		140.3		140.0		140.7
14		142.7		142.7		144.3
15	4.30 (1H, d, 7.8)	81.7	4.27 (1H, d, 7.6)	82.1	4.50 (1H, d, 7.8)	82.5
16a	2.23 (1H, m)	37.8	2.56 (1H, m)	38.0	2.58 (1H, m)	37.9
16b	1.50 (1H, m)		1.58 (1H, m)		1.65 (1H, m)	
17	3.21 (1H, m)	46.5	3.22 (1H, m)	46.6	3.43 (1H, m)	47.2
18	1.73 (3H, s)	16.2	1.75 (3H, s)	16.3	1.78 (3H, s)	16.1
19	0.97 (3H, s)	15.8	0.99 (3H, s)	16.5	0.87 (3H, s)	16.5
20		128.5		128.6		128.6

21	7.21 (1H, br s)	138.8	7.21 (1H, br s)	138.9	7.32 (1H, br s)	139.1
22	6.32 (1H, br s)	110.2	6.34 (1H, br s)	110.3	6.44 (1H, br s)	110.6
23	7.27 (1H, br s)	142.9	7.28 (1H, br s)	142.6	7.28 (1H, br s)	142.8
28a	3.49(1H, d, 7.6)	77.8	3.52 (1H, d, 7.4)	77.9	3.68(1H, d, 7.5)	78.0
28b	3.41 (1H, d, 7.6)		3.39 (1H, d, 7.4)		3.58 (1H, d, 7.5)	
29	1.15 (3H, s)	19.5	1.16 (3H, s)	19.4	1.17 (3H, s)	19.6
30	1.40 (3H, s)	20.7	1.43 (3H, s)	21.0	1.36 (3H, s)	20.5
12-O-Et						
1’	3.54 (1H, m)	63.6	3.79 (1H, m)	63.8	3.76 (1H, m)	64.0
	3.13 (1H, m)		3.35 (1H, m)		3.53 (1H, m)	
2’	1.13 (3H, t, 7.1)	15.5	0.99 (3H, t, 7.0)	14.7	1.23 (3H, t, 7.1)	15.1
OAc$_1$						
Ac$_1$-CO		169.9		169.9		171.5
Ac$_1$-Me	1.99 (3H, s)	20.8	1.92 (3H, s)	20.8	2.12 (3H, s)	21.6
OAc$_2$						
Ac$_2$-CO		169.7				
Ac$_2$-Me	2.07 (3H, s)	21.6				
OTig$_1$						
1’’		166.4		166.9		
2’’		128.8		128.7		
3’’	6.92 (1H, dd, 1.2, 7.0)	136.6	6.83 (1H, dd, 1.2, 7.0)	136.2		
2’’-CH$_3$	1.90 (3H, s)	12.1	1.88 (3H, s)	12.3		
3’’-CH$_3$	1.77 (3H, d, 7.0)	14.3	1.79 (3H, d, 7.0)	14.3		
OTig$_2$						
1’’’				166.8		
2’’’				129.5		
3’’’			6.95 (1H, dd, 1.2, 7.0)	136.5		
2’’’-CH$_3$			1.96 (3H, s)	12.4		
3’’’-CH$_3$			1.78 (3H, d, 7.0)	14.2		

Compounds **1-3** were tested for their antibacterial activity against oral pathogens *Streptococcus mutans* ATCC 25175 and *Porphyromonas gingivalis* ATCC 33277. Compound **3** exhibited significant antibacterial activity against the oral pathogen, *Porphyromonas gingivalis* ATCC 33277, with an MIC value of 31.25 µg/mL. All the compounds showed no activity against *S. mutans* ATCC 25175.

EXPERIMENTAL

General Experimental Procedures. Optimal rotations were measured on a Perkin-Elmer PE 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 16 PC FT-IR spectrophotometer. 1D and 2D

NMR spectra were performed on a Bruker AV400 spectrometer, with TMS as an internal standard. HRESIMS were recorded on a PE Biosystems Mariner System 5140 LC/MS spectrometer. Column chromatography was carried out using silica gel (Merck) and Sephadex LH-20 (Pharmacia; Uppsala, Sweden). Preparative HPLC was carried out on a Waters Prep LC 4000 system with a UV detector and an X-bridge C-18 column (19×150 mm, 5 μm). Fractions were visualized by heating silica gel plates sprayed with 4-(dimethylamino)benzaldehyde.

Plant Materials. The dried fruits of *M. toosendan* were collected from Wanxian, Sichuan Province, People's Republic of China, in July 2010, and identified by Prof. Qin Minjian (Department of Natural Medicinal Resources, China Pharmaceutical University, Nanjing, China). A voucher specimen (No. 28-78-56-3) was deposited in the herbarium of China Pharmaceutical University.

Extraction and Isolation. The air-dried fruits of *M. toosendan* (20 kg) were crushed and extracted with EtOAc (40 L, 2 h each) under reflux three times. All the extracts were combined and concentrated under vacuum to give a residue (Fraction A, 300 g). Fraction A was subjected to silica gel column chromatography (CC, 13×85 cm, 230-400 mesh), eluted with a gradient of petroleum ether-EtOAc (9:1, 8:2, 7:3 and 6:4, each 18000 mL) to afford 20 fractions (Frs. 1-20). Fr. 11 (7.2 g) was further divided into 7 subfractions (Frs. 11.1-11.7) by silica gel CC (4×42 cm, 230-400 mesh), using $CHCl_3$-Me_2CO (100:2, 100:4 and 100:6, each 1500 mL) as the eluent. Fr. 11.1 (3.0 g) was further chromatographed on silica gel CC (4×42 cm, 230-400 mesh), eluting with a gradient of petroleum ether-Me_2CO (90:10, 88:12, 85:15 and 80:20, each 1500 mL) to afford Frs. 11.1.1-11.1.9. Fr. 11.1.3 (0.6 g) was chromatographed on Sephadex LH-20 [2×80 cm, $CHCl_3$-MeOH (1:1), 200 mL] to afford Frs. 11.1.3.1-11.1.3.6. Fr. 11.1.3.4 (36 mg) was purified by reversed phase preparative HPLC using a gradient of increasing MeCN (55-85%) in H_2O at 18 mL/min for 20 min to give **1** (t_R=10.2 min, 10 mg) and **2** (t_R=12.6 min, 8 mg). Frs. 15 and 16 (13 g) were chromatographed on silica gel CC (4×42 cm, 230-400 mesh), eluting with a gradient of $CHCl_3$-Me_2CO (100:2, 100:4, 100:6, 100:8 and 100:10, each 2000 mL) to afford Frs. 15.1-15.8. Fr. 15.5 (2.5 g) was chromatographed on silica gel CC (3×39 cm, 230-400 mesh), eluting with a gradient of petroleum ether-Me_2CO (75:25, 70:30, 60:40 and 1:1, each 600 mL) to afford Frs. 15.5.1-15.5.8. Fr. 15.5.5 (32 mg) was purified by reversed phase preparative HPLC using a gradient of increasing MeCN (35-55%) in H_2O at 18 mL/min for 20 min to yield **3** (t_R=9.7 min, 6 mg).

Compound 1: amorphous powder; $[\alpha]^{25}_D$ -6.3 (c 0.08, $CHCl_3$); IR (KBr) ν_{max} 3417, 2924, 1737, 1452, 1256, 1051 cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$) data, see Table 1; HRESIMS *m/z* 677.3305 $[M+Na]^+$ (calcd for $C_{37}H_{50}O_{10}Na$, 677.3302).

Compound 2: amorphous powder; $[\alpha]^{25}_D$ +8.6 (c 0.13, $CHCl_3$); IR (KBr) ν_{max} 2924, 1494, 1051 cm^{-1}; ^{1}H- and ^{13}C-NMR ($CDCl_3$) data, see Table 1; HRESIMS *m/z* 717.3619 $[M+Na]^+$ (calcd for $C_{40}H_{54}O_{10}Na$, 717.3615).

Compound 3: amorphous powder; $[\alpha]^{25}_D$ +9.5 (c 0.11, $CHCl_3$); IR (KBr) ν_{max} 3445, 2928, 1719, 1245, 1056 cm^{-1}; ^{1}H- and ^{13}C-NMR ($CDCl_3$) data, see Table 1; HRESIMS *m/z* 553.2876 $[M+Na]^+$ (calcd for $C_{30}H_{42}O_8Na$, 553.2879).

MIC determinations.

The MIC values of all compounds against selected oral bacteria were determined using liquid cultures in 96-well culture plates according to a modification of the method described by Shapiro et al.[12] Triclosan (Ciba Speciality Chemicals; Shanghai, China) was used as positive control. Trypticase soy broth (TSA, Becton-Dickinson Microbiology Systems, Cockeysville, MD, USA) was used for *Streptococcus mutans* and *Porphyromonas gingivalis*. Todd Hewitt broth supplemented with 1% yeast extract (Difco Laboratories, Detroit, MI) was used. Serial dilutions (1.0-0.002%) of each extract and compound were prepared in each culture medium. Aliquots (200 μL) of each dilution were dispensed in 96-well cell culture plates (Becton-Dickinson Microbiology Systems). Subsequently, 10^5-10^6 test bacteria that had been cultured overnight in each culture medium were inoculated into each well and cultured for 1-2 days under anaerobic conditions. Then the absorbance was measured at 630 nm (Bio-tek, ELX808; Winooski, VT, USA). The highest dilution at which no growth (OD630 $\leq$ 0.05) was observed was defined as the minimum inhibitory concentration (MIC).

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REFERENCES

1. S.-K. Chen, B.-Y. Chen, and H. Li, 'Flora Republicae Popularis Sinicae (Zhongguo Zhiwu Zhi)', Science Press, Beijing, 1997, **43**, pp. 102-103.
2. M. Nakatani, *Heterocycles*, 1999, **50**, 595.
3. K. Tada, M. Takido, and S. Kitanaka, *Phytochemistry*, 1999, **51**, 787.
4. Q. Zhang, Y. Shi, X.-T. Liu, J.-Y. Liang, N. Y. Ip, and Z.-D. Min, *Planta Med.*, 2007, **73**, 1298.

5. Y.-S. Sang, C.-Y. Zhou, A.-J. Lu, X.-J. Yin, Z.-D. Min, and R.-X. Tan, *J. Nat. Prod.*, 2009, **72**, 917.
6. Q.-G. Tan and X.-D. Luo, *Chem. Rev.*, 2011, **111**, 7437.
7. G.-Y. Zhu, L.-P. Bai, L. Liu, and Z.-H. Jiang, *Phytochemistry*, 2014, **107**, 175.
8. S. Su, L.-Q. Shen, Y. Zhang, J.-Y. Liu, J.-N. Cai, L.-L. Hao, Y.-L. Feng, and S.-L.Yang, *Phytochem. Lett.*, 2013, **6**, 418.
9. Q. Zhang, J.-K. Li, R. Ge, J.-Y. Liang, Q.-S. Li, and Z.-D. Min, *Fitoterapia*, 2013, **90**, 192.
10. L. Zhao, C.-H. Huo, L.-R. Shen, Y. Yang, Q. Zhang, and Q.-W. Shi, *Chem. Biodivers.*, 2010, **7**, 839.
11. J.-F. Hu, H. Fan, L.-J. Wang, S.-B. Wu, and Y. Zhao, *Phytochem. Lett.*, 2011, **4**, 292.
12. S. Shapiro, A. Meier, and B. Guggenheim, *Oral Microbiol. Immunol.*, 1994, **9**, 202.