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Limonoids from the fruits of *Melia toosendan*

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ABSTRACT

Four (**1**–**4**) new and seven known limonoids were isolated from the EtOH extract of the fruits of *Melia toosendan*. The structures of the new compounds were established on the basis of spectroscopic methods to be 12-*O*-methyl-1-*O*-deacetylnimbolinin B (**1**), 12-*O*-methy-1-*O*-tigloyl-1-*O*-deacetylnimbolinin B (**2**), 12-*O*-ethylnimbolinin B (**3**), and 1-*O*-cinnamoyl-1-*O*-debenzoylohchinal (**4**). Additionally, two new tirucallane-type triterpenoids, named meliasenins S (**5**) and T (**6**), were obtained from the same fractions during purification of the limonoids.

1. Introduction

Limonoids are a class of highly oxygenated tetranortriterpenoids and are typical metabolites of the plants belonging to the genus *Melia* (Meliaceae) (Roy and Saraf, 2006; Zhao et al., 2010). Although *Melia* plants have been extensively investigated, it is still an active area of research for natural product chemists. In an earlier study on the fruits of *Melia toosendan* Sieb. et Zucc., we have reported a number of cytotoxic triterpenoids and steroids (Wu et al., 2010). A further effort to search for new limonoids from the fruits led to the isolation of eleven limonoids along with two tirucallane-type triterpenoids. In this letter, we deal with the isolation and structure elucidation of the new compounds (**1**–**6**). The biogenetic relationship among the various limonoids, tirucallane-type and euphane-type triterpenoids isolated from the fruits is briefly discussed herein.

2. Results and discussion

Our earlier phytochemical investigation on the EtOH extract of the dried fruits yielded ten fractions (Fr.1–Fr.10). Fr.3 and Fr.6 were found to afford euphane-type and tirucallane-type triterpenoids, whereas Fr.2, Fr.8 and Fr.10 furnished steroids only (Wu et al., 2010). The remained Fr.1, Fr.4 and Fr.5 did not show any interesting spots on TLC analysis, but both Fr.7 and Fr.9 disclosed the presence of a variety of limonoids from the characteristic color on TLC upon treatment with Ehrlich's reagent (Bennett and Hasegawa, 1981). After comprehensive isolation and purification using column chromatography (CC), Sephadex LH-20 and semi-preparative HPLC, four (**1**–**4**) new and seven known limonoids together with two new tirucallane-type triterpenoids (**5**, **6**) (Fig. 1) were obtained from Fr.7 and Fr.9. Comparing their MS and NMR data and their physical properties with literature, the known compounds were ultimately identified as 12-*O*-ethyl-1-deactylnimbolinin B (**7**) (Xie et al., 2008), nimbolinin D (**8**) (Nakatani et al., 2000), nimbolinin B (**9**) (Kraus and Bokel, 1981), 12-*O*-methylnimbolinin B (**10**) (Mulholland et al., 1998), 1-*O*-tigloyl-1-*O*-debenzoylohchinal (**11**) (Xie et al., 2008), 1-*O*-cinnamoyltrichilinin (**12**) (Rajab and Bentley, 1988), and 1-*O*-tigloyltrichilinin (also named trichilinin B) (**13**) (Rajab and Bentley, 1988; Zhou et al., 1995).

The molecular formula of **1** was determined as $C_{34}H_{46}O_9$ by HR-ESIMS, which showed an $[M+Na]^+$ ion peak at *m*/*z* 621.3040. The ^1H NMR spectrum (Table 1) showed eight methyl groups (δ 0.91, 1.13, 1.44, 1.77, 1.81, 1.89, 2.04, and 3.40), one oxygenated methylene group resonating at δ 3.48 and 3.55 (ABq, J = 7.4 Hz), six oxygenated methine protons (δ 3.60, 4.11, 4.80, 4.88, 4.93, 5.71), and one olefinic proton (δ 6.92) in addition to three characteristic olefinic protons [δ 6.37 (d, J = 1.5 Hz), 7.24 (s), 7.30 (d, J = 1.5 Hz)] attributed to a β-substituted furan ring (Xie et al., 2008; Tada et al., 1999). The ^{13}C NMR (DEPT) spectrum of **1** (Table 2) revealed the presence of thirty-four carbons classified as eight methyls (one oxygenated at δ 54.6), four methylenes (one oxygenated at δ 78.0), nine sp^3 methines (six oxygenated at δ 70.9, 72.2, 72.6, 74.7, 77.6, and 98.5) and four sp^2 methines (δ 110.4, 136.8, 139.0, and 142.7), three sp^3 quaternary carbons and four sp^2 quaternary carbons (δ

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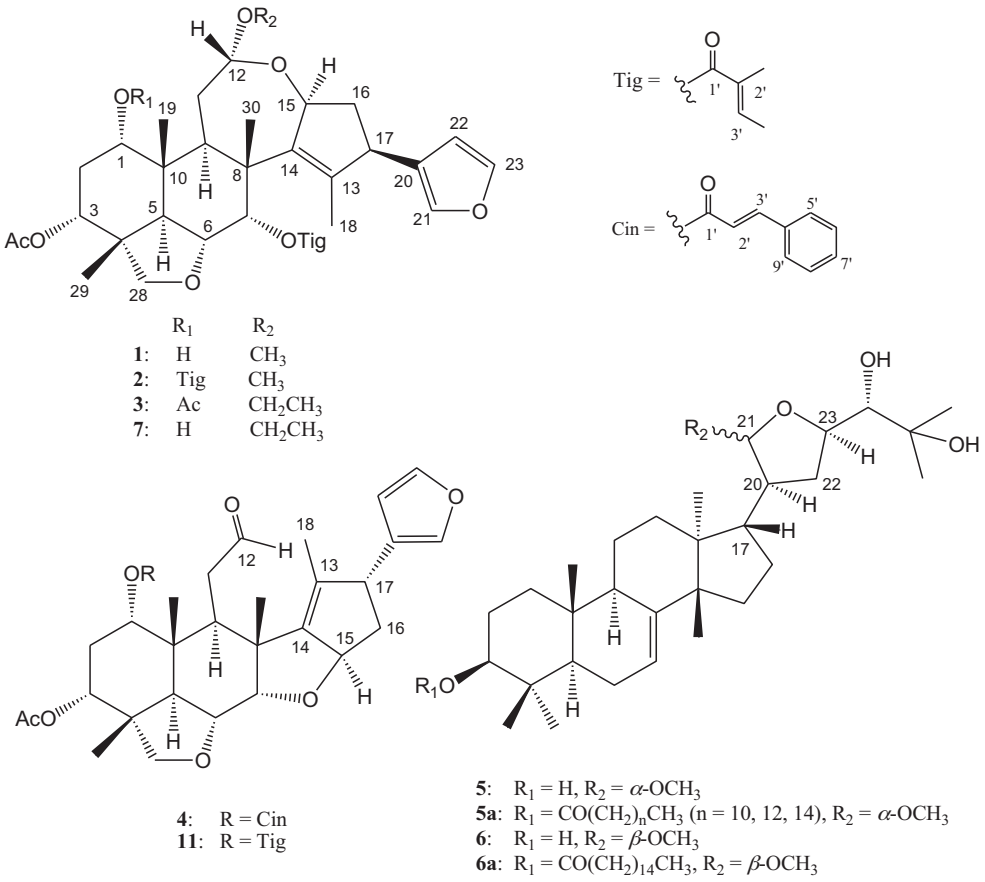

Fig. 1. Chemical structures of new compounds **1**–**6**.

128.2, 128.8, 142.3, and 142.8), as well as two carbonyl carbons at δ 166.6 and 170.8 (Table 2). The above NMR data of **1** showed general features very similar to those of the known limonoid 12-*O*-ethyl-1-deactylnimbolinin B (**7**) previously also isolated from the fruits of *M. toosendan* (Xie et al., 2008). The only difference between **1** and **7** (Fig. 1) was that the ethoxy group at C-12 in **7** was replaced by a methoxy in **1**. The esterification positions of the acetyl (at C-3) and the tigloyl (at C-7) were confirmed by the HMBC NMR experiment (Fig. 2). The relative configuration of **1** was consistent with that of **7** according to the proton coupling patterns (Table 1) and was substantially secured by the NOESY NMR experiment. The small coupling constants observed for H-1 (J = 2.5 Hz), H-3 (J = 2.9 Hz) and H-7 (J = 2.7 Hz) indicated that they were all β-orientated. Meanwhile, clear NOE correlations (Fig. 2) were observed between H-1/H-3, H-3/Me-29, Me-29/H-6, H-6/H-7, H-7/Me-30, Me-30/Me-19, and Me-19/H-1, 1-OH (δ 4.24)/H-9, H-9/H-15, H-15/H-17, and H-15/12-OMe. Therefore, **1** was determined to be 12-*O*-methyl-1-*O*-deacetyl-nimbolinin B.

The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of compound **2** closely resembled those of **1**, except for an additional tigloyl unit at C-1 [δ_H 1.84 (CH_3), 1.99 (CH_3), 6.99 (CH); δ_C 12.4, 14.2, 129.3, 136.5, 166.9]. This was supported by the fact that H-1 in **2** was shifted downfield to δ 4.85 (br s) compared to that of in **1** (δ 3.60). Like **1**, the esterification positions and the relative configuration in **2** were confirmed by 2D NMR experiments (COSY, HSQC, HMBC and NOESY). Obviously, a ^3J HMBC correlation was observed between

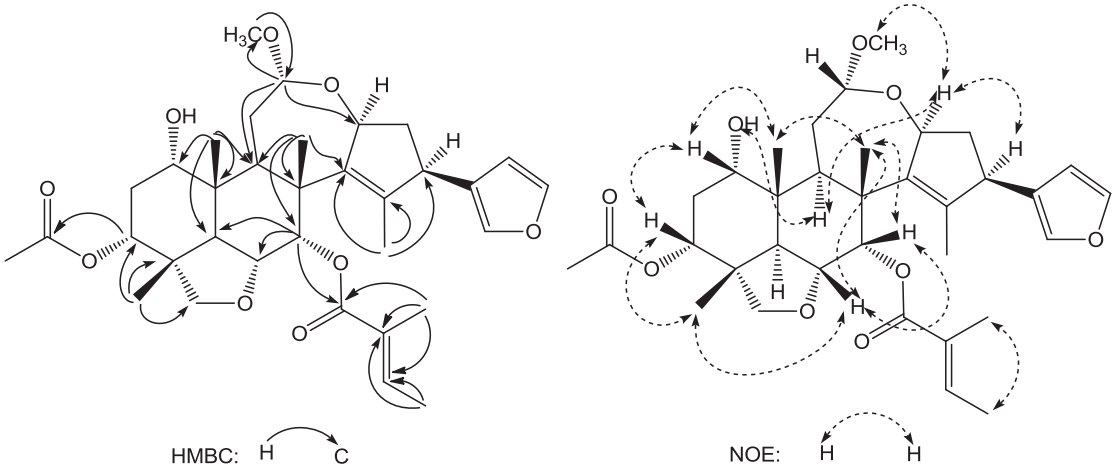

Fig. 2. Key HMBC and selected NOESY correlations of **1**.

Table 1
^{1}H NMR data of compounds **1**–**4** ($CDCl_3$, 500 MHz, *J* values in Hz).[a]

No.	1	2	3	4
1	3.60, 1H, t, 2.5	4.85, 1H, br s	4.74, 1H, br s	4.92, 1H, br s
2	2.17, 2H, m	2.13, 2.24, each 1H, m	2.18, 2H, m	2.43, 2H, m
3	4.88, 1H, t, 2.9	4.96, 1H, br s	4.94, 1H, br d, 2.6	5.00, 1H, br s
5	2.81, 1H, d, 12.8	2.76, 1H, d, 12.9	2.82, 1H, d, 12.8	2.88, 1H, d, 12.6
6	4.11, 1H, dd, 12.8, 2.7	4.13, 1H, dd, 12.9, 2.5	4.09, 1H, dd, 12.8, 2.6	4.03, 1H, dd, 12.6, 2.8
7	5.71, 1H, d, 2.7	5.86, 1H, d, 2.5	5.80, 1H, d, 2.6	4.23, 1H, d, 2.8
9	3.02, 1H, br d, 6.2	3.14, 1H, d, 10.3	3.15, 1H, d, 10.5	2.74, 1H, d, 8.6
11	1.76, 2H, m	1.60, 2.28, each 1H, m	1.58, 1.81, each 1H, m	2.25, 2.30, each 1H, m
12	4.80, br s	4.61, 1H, br s	4.78, 1H, br s	9.14, 1H, d, 5.0
15	4.93, 1H, br d, 7.9	4.89, 1H, d, 7.7	4.99, 1H, d, 7.8	5.43, 1H, br d, 7.3
16	1.60, 1H, br d	1.56, 1H, m	1.54, 1H, m	2.24, 1H, m
	2.24, 1H, m	1.79, 1H, m	2.21, 1H, m	2.21, 1H, m
17	3.28, 1H, br d, 9.4	3.27, 1H, d, 8.8	3.26, 1H, d, 9.4	3.63, 1H, d, 7.5
18	1.77, 3H, br s	1.76, 3H, s	1.76, 3H, s	1.58, 3H, s
19	0.91, 3H, s	1.00, 3H, s	0.99, 3H, s	1.01, 3H, s
21	7.24, 1H, s	7.23, 1H, s	7.23, 1H, s	7.03, 1H, s
22	6.37, 1H, d, 1.5	6.37, 1H, br s	6.35, 1H, br s	5.96, 1H, br s
23	7.30, 1H, d, 1.5	7.29, 1H, br s	7.30, 1H, br s	7.09, 1H, br s
28	3.48, 1H, d, 7.4	3.46, 1H, d, 7.4	3.43, 1H, d, 7.5	3.63, 1H, d, 7.5
	3.55, 1H, d, 7.4	3.52, 1H, d, 7.4	3.51, 1H, d, 7.5	3.78, 1H, d, 7.5
29	1.13, 3H, s	1.18, 3H, s	1.17, 3H, s	1.25, 3H, s
30	1.44, 3H, s	1.46, 3H, s	1.43, 3H, s	1.29, 3H, s
1-OAc			2.10, 3H, s	
3-OAc	2.04, 3H, s	1.94, 3H, s	2.01, 3H, s	1.96, 3H, s
Tig				
3′	6.92, 1H, m	6.89, 1H, qq, 7.2, 1.0	6.95, 1H, qq, 7.2, 1.0	
3″		6.99, 1H, qq, 7.2, 1.5		
2′-Me	1.89, 3H, br s	1.89, 3H, br s	1.92, 3H, br s	
2″-Me		1.99, 3H, br s		
3′-Me	1.81, 3H, br d, 7.1	1.80, 3H, br d, 7.2	1.81, 3H, br d, 7.2	
3″-Me		1.84, 3H, br d, 1.5		
Cin				
2′				6.41, 1H, d, 16.2
3′				7.78, 1H, d, 16.2
Ph				7.39–7.49, 5H, m
1-OH	4.24, 1H, br s			
12-OMe	3.40, 3H, s	3.05, 3H, s		
12-OEt			3.56, 2H, m, 1.16, 3H, d, 7.1	

[a] Assignments were made by a combination of 1D and 2D NMR (COSY, HSQC, HMBC) experiments.

H-1 and the ester carbonyl carbon (C-1″) at δ 166.9. Thus, compound **2** was determined to be 12-*O*-methy-1-*O*-tigloyl-1-*O*-deacetylnimbolinin B.

Compound **3**, possessing an ethoxy group at C-12 [δ_H 3.56 (2H, m), 1.16 (2H, d, J = 7.1 Hz); δ_C 62.4 (t), 14.8 (q)], has general NMR features (Tables 1 and 2) very similar to those of 12-*O*-ethyl-1-deactylnimbolinin B (**7**) (Xie et al., 2008). The only difference between **3** and **7** was that **3** contained an additional acetyl group at C-1 [δ_H 2.10 (3H, s); δ_C 169.2 (s), 21.2 (q)]. Detailed analysis of 2D NMR spectra (COSY, HSQC, HMBC and NOESY) of **3** refined the proposed structure. Consequently, compound **3** was established as 12-*O*-ethyl-nimbolinin B.

Compound **4** gave an $[M+Na]^+$ ion peak at *m*/*z* 637.2783 in the positive mode HR-ESIMS, indicating its molecular formula to be $C_{37}H_{42}O_8$. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **4** closely resembled those of 1-*O*-tigloyl-1-*O*-debenzoylohchinal (**11**) (Xie et al., 2008). The only difference between **4** and **11** (Fig. 1) was that the tigloyl group at C-1 in **11** was replaced by a cinnamoyl moiety [δ_H 6.41, 7.78 (each 1H, d, J = 16.2 Hz), 7.39–7.50 (5H, m); δ_C 117.8, 128.1 × 2, 129.1 × 2, 130.8, 133.8, 145.7, 165.3] in **4**. Both compounds **4** and **11** have an aldehyde group at C-12. Similar to compounds **1**–**3**, the esterification positions and the relative configuration in **4** were ascertained by detailed 1D and 2D NMR experiments. Notably, a ^3J HMBC correlation was observed between H-1 (δ 4.92) and the carbonyl carbon (δ 165.3) of the cinnamoyl group. Therefore, **4** was determined to be 1-*O*-cinnamoyl-1-*O*-debenzoylohchinal.

Both meliasenin S (**5**) and meliasenin T (**6**) were found to have the same molecular formula of $C_{31}H_{52}O_5$. The ^{1}H NMR spectrum (Table 3) of **5** exhibited signals assignable to seven tertiary methyl groups (δ 0.75, 0.86, 0.86, 0.97, 0.98, 1.27 and 1.30), one methoxyl group at δ 3.35 (3H, s), and one olefinic proton at δ 5.26 (1H, br d, J = 3.1 Hz). The ^{13}C NMR and DEPT spectra of **5** displayed thirty carbon signals classified as seven methyl carbons, eight sp^3 methylene carbons, eight sp^3 and one sp^2 (δ 118.2) methine carbons, and five sp^3 and one sp^2 (δ 145.4) quaternary carbons in addition to a methoxyl carbon resonating at δ 55.6. These NMR data resembled those of meliasenin R (**5a**), a tirucallane-type triterpenoid previously isolated from the same extract of the fruits of *M. toosendan* (Wu et al., 2010). The obvious difference between these two compounds was that the ester moiety at C-3 in **5a** was absent in **5** (Fig. 1). This was supported by its IR spectrum (double bond: 1621 cm^{-1}, hydroxyl: 3464 cm^{-1}; but no typical ester absorption band). The β-orientation of the 3-OH group was indicated from the characteristic splitting pattern (dd, J = 11.6, 3.0 Hz) of H-3 resonating at δ 3.26. The relative stereochemistry at **5** was then assigned as depicted by comparing the corresponding ^{1}H and ^{13}C NMR data with those of **5a**. Notably, the chemical shift (δ 109.0) of C-21 in **5** was in good agreement with the presence of an α-OMe group at C-21 (Wu et al., 2010; Xie et al., 2007). Meanwhile, the ^{1}H and ^{13}C NMR data (Table 3) of **6** were quite similar to those of **5**. The only difference was the chemical shift of C-21 (**6**: δ 104.9, **5**: δ 109.0), which suggested that **6** was the 21-epimer of **5** (Wu et al., 2010; Xie et al., 2007). Therefore, **5** and **6** were defined as 21,23-epoxy-21α-methoxy-tirucall-7-en-3β,24α,25-triol and 21,23-epoxy-21β-methoxy-tirucall-7-en-3β,24α,25-triol, respectively. Compounds **5** and **6** could be regarded as the deesterified derivative of meliasenin R (**5a**) and

Table 2
^{13}C NMR data of compounds **1**–**4** (in $CDCl_3$, 125 MHz).[a]

No.	1	2	3	4	No.	1	2	3	4
1	70.9	70.9	70.8	71.7	1-OAc			169.2	
2	29.0	28.1	27.2	28.0				21.2	
3	72.6	71.6	71.2	71.3	3-OAc	170.8	169.9	169.3	170.4
4	42.3	42.5	42.0	42.7		21.2	20.8	20.4	21.2
5	38.9	41.0	39.5	40.1	*Tig*				
6	72.2	72.3	71.8	72.7	1′	166.6	166.9	165.8	
7	74.7	74.5	74.1	85.7	2′	128.8	128.9	127.9	
8	45.2	45.4	44.9	48.7	3′	136.8	136.1	136.1	
9	36.3	37.0	35.4	38.1	2′-Me	12.1	12.3	11.6	
10	41.1	40.6	39.9	40.6	3′-Me	14.5	14.4	13.9	
11	30.6	31.5	31.5	40.6	1″		166.9		
12	98.5	98.0	96.1	199.2	2″		129.3		
13	142.3	140.4	139.8	136.6	3″		136.5		
14	142.8	142.7	142.3	145.4	2″-Me		12.4		
15	77.6	76.5	76.5	88.1	3″-Me		14.2		
16	37.9	37.9	37.4	41.7	*Cin*				
17	46.7	46.7	45.8	49.5	1′				165.3
18	16.2	16.2	15.6	13.5	2′				117.8
19	16.4	16.5	15.3	15.5	3′				145.7
20	128.2	128.4	128.3	126.6	4′				133.8
21	139.0	139.0	138.5	138.3	5′, 9′				128.1
22	110.4	110.5	109.9	109.8	6′, 8′				129.1
23	142.7	142.7	142.2	143.2	7′				130.8
28	78.0	77.9	77.5	77.7	12-OMe	54.6	54.0		
29	19.0	19.1	18.8	19.5	12-OCH_2CH_3			62.4	
30	20.6	21.1	20.1	16.9	12-OCH_2CH_3			14.8	

[a] Assignments were made by a combination of 1D (DEPT) and 2D NMR (COSY, HSQC, HMBC) experiments.

meliasenin Q (**6a**) (Fig. 1), respectively; however, they were detected in the crude EtOH extract by TLC and HPLC analyses, suggesting that they were not purification artifacts. Interestingly, such 21-epimers possessing an α-OH group at C-3 also co-occurred in another Meliaceae plant *Aglaia duperreana* (Xie et al., 2007).

Meliaceae plants have proven to be a rich source of limonoids, and some exhibited considerable biological activities (Roy and Saraf, 2006; Zhao et al., 2010). The above isolated limonoids **1**–**4** and **7**–**13** have been deposited in our Natural Products Pool (NPP) for future biological evaluation via high-throughput screening

Table 3
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **5** and **6** (in $CDCl_3$).[a]

No.	5		6	
	δ_H (J value in Hz)	δ_C	δ_H (J value in Hz)	δ_C
1	1.12 m, 1.66 m	37.2	1.12 m, 1.66 m	37.2
2	1.58 m, 1.65 m	27.7	1.58 m, 1.65 m	27.7
3	3.26 dd (11.6, 3.0)	79.2	3.24 dd (11.6, 3.0)	79.2
4		38.9		38.9
5	1.28 dd (12.1, 5.7)	50.7	1.28 dd (12.1, 5.7)	50.7
6	1.98 m, 2.16 m	23.9	1.97 m, 2.10 m	23.8
7	5.26 br d (3.1)	118.2	5.27 br d (2.7)	118.2
8		145.4		145.5
9	2.28 m	48.8	2.23 m	48.8
10		35.0		34.9
11	1.50 m, 1.57 m	17.6	1.50 m, 1.58 m	17.7
12	1.51 m, 1.72 m	31.7	1.31 m, 1.86 m	31.2
13		43.7		43.5
14		50.9		50.7
15	1.71 m, 1.92 m	34.4	1.49 m, 1.51 m	34.2
16	1.24 m, 1.88 m	27.4	1.22 m, 1.86 m	27.3
17	1.76 m	50.3	2.01 m	45.0
18	0.86 s	22.6	0.84 s	23.2
19	0.75 s	13.1	0.75 s	13.1
20	2.10 m	47.8	1.99 m	46.3
21	4.79 d (2.8)	109.0	4.70 d (3.3)	104.9
22	1.50 m	33.8	1.88 m	31.6
23	4.22 br dt (10.2, 4.2)	76.7	4.42 br dt (9.1, 1.9)	78.8
24	3.24 d (8.4)	75.4	3.17 br s	76.7
25		73.1		72.9
26	1.27 s	26.4	1.26 s	26.3[b]
27	1.30 s	26.4	1.27 s	26.4[b]
28	0.86 s	27.6	0.85 s	27.7
29	0.97 s	14.7	0.97 s	14.7
30	0.98 s	27.1	0.98 s	27.2
OMe	3.35 s	55.6	3.36 s	55.2

[a] Assigments were made by NMR experiments and comparison with the data of meliasenin R (**5a**) and meliasenin Q (**6a**) (Wu et al., 2010).
[b] Assignments may be interchangeable.

(HTs). From the biosynthetic point of view, the co-occurrence of different limonoids together with tirucallane-type and/or euphane-type triterpenoids is interesting. Tirucallane-type triterpenoids (**5**, **6**) may be the ultimate biogenetic precursors of trichilinins (**12**, **13**), and the later ones could be further considered to be the biogenetic precursors of C-seco (ring C-oxidized) limonoids such as nimbolinins (**1**–**3**, **7**–**10**) and ohchinols (**4**, **11**) (Roy and Saraf, 2006; Zhou et al., 1995).

3. Experimental

3.1. General experimental procedures

Optical rotations were determined using a PerkinElmer 341 polarimeter. IR spectra were measured on a Thermo Nicolet NEXUS-670 FT-IR spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer. Chemical shifts are expressed in δ (ppm), and are referenced to the residual non-deuterated solvent signals. Electrospray ionization mass spectra (ESI-MS) were measured on a Bruker Daltonics micrOTOF-QII mass spectrometer. Analytical and semi-preparative HPLC was performed on a Beckman System consisting of a Beckman Coulter System Gold 508 autosampler, Gold 126 gradient HPLC pumps, a Beckman System Gold 168 UV detector and a Sedex 80 (SEDERE, France) evaporative light-scattering detector (ELSD). A Benetnach C18 column (250 mm × 10 mm, i.d.; 5 μm; Jiangsu Hanbon Science & Technology Co., Ltd., China) was utilized. Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Ji-Yi-Da Silysia Chemical Ltd., China) or Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). Silica gel-precoated plates (GF_{254}, 0.25 mm, Yantai Kang-Bi-Nuo Silysia Chemical Ltd, China) were used for TLC. Spots were visualized under UV light (254/365 nm) and/or by spraying with 5% (v/v) H_2SO_4–EtOH followed by heating to 120 °C. The solvents for CC were analytical grade (Shanghai Chemical Reagents Co., Ltd., China) and those for HPLC were HPLC grade (Jiangsu Hanbon Science & Technology Co., Ltd., China).

3.2. Plant material

Dried fruits of *M. toosendan* were purchased from Shanghai Jiu-Zhou-Tong Medicine Co., Ltd. and were originally collected in October 2007 from Wanyuan Country, Sichuan Province of China. The plant was identified by Prof. Jian-Wei Chen (College of Pharmacy, Nanjing University of Traditional Chinese Medicine). A voucher specimen (no. 100310) was deposited at the Herbarium of the Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University.

3.3. Extraction and isolation of compounds 1–13

The dried fruits were extracted with 95% EtOH. The solvent was removed under vacuum to give a crude extract, which was then suspended in H_2O and exhaustively extracted with EtOAc. The EtOAc extract was subjected to CC over silica gel with a petroleum ether (PE)–acetone gradient to yield ten fractions (Fr.1–Fr.10) (Wu et al., 2010). Fr.7 (PE–acetone 2:1, v/v, 15.8 g) was further separated by silica gel column with a CH_2Cl_2–MeOH gradient (24:1–15:1, v/v) to afford seven subfractions (Fr.7A–Fr.7G). Compounds **2** (9.0 mg) and **3** (10.1 mg) were isolated from Fr.7A by semi-preparative HPLC (MeOH–H_2O 74:26, v/v; flow rate: 3.0 mL/min). Fr.7F, eluted with a PE–EtOAc gradient (2:1–1:1, v/v), was subsequently purified by HPLC with MeOH–H_2O (75:25, v/v) to furnish compounds **1** (15.2 mg), **7** (10.6 mg) and **10** (11.1 mg). Fr. 9 (PE–acetone 3:2, v/v, 6.8 g) was subjected to CC over silica gel with a gradient of CH_2Cl_2–EtOAc (8:1–4:1, v/v) to furnish six subfractions (Fr.9A–Fr.9F). Fr.9B was rechromatographed on silica gel column with PE–EtOAc (6:1, v/v) and subsequently subjected to HPLC (ACN–H_2O 70:30, v/v) to generate compounds **5** (4.5 mg) and **6** (2.5 mg). Fr.9C was purified by silica gel column (PE–EtOAc 5:1, v/v) followed by gel permeation chromatography on Sephadex LH-20 (in MeOH) to give compound **8** (18.5 mg). Fr.9D was subjected to CC over silica gel (PE–EtOAc 1:1, v/v) and subsequently refined by semi-preparative HPLC to afford compounds **12** (5.2 mg; ACN–H_2O 69:31, v/v) and **13** (9.9 mg; MeOH–H_2O 78:22, v/v). Fr.9E yielded compounds **4** (20.1 mg) and **11** (10.2 mg) after purification by silica gel column (CH_2Cl_2–EtOAc 2:1, v/v) and Sephadex LH-20 (in MeOH). Compound **9** (13.2 mg) was obtained from Fr.9F by silica gel CC with PE–EtOAc (1:1, v/v) and further purified by C-18 HPLC with MeOH–H_2O (65:35, v/v).

3.3.1. 12-O-Methyl-1-O-deacetylnimbolinin B (1)

White amorphous powder, $[\alpha]_D^{22}$: −34.0 (*c* 0.10, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}): 3413 (br), 2929, 2858, 1724, 1634, 1571, 1412, 1257, 1157, 1080, 1054, 870; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESIMS *m/z* 621.3040 $[M+Na]^+$ (calcd for $C_{34}H_{46}O_9Na$, 621.3034).

3.3.2. 12-O-Methy-1-O-tigloyl-1-O-deacetylnimbolinin B (2)

White amorphous powder, $[\alpha]_D^{22}$: −66.0 (*c* 0.13, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}): 3396 (br), 2935, 1724, 1720, 1600, 1576, 1442, 1424, 1385, 1345, 1270, 1115, 1044, 1013, 872; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESIMS *m/z* 703.3441 $[M+Na]^+$ (calcd for $C_{39}H_{52}O_{10}Na$, 703.3453).

3.3.3. 12-O-Ethylnimbolinin B (3)

White amorphous powder, $[\alpha]_D^{22}$: −26.0 (*c* 0.13, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}): 3410 (br), 2937, 1727, 1721, 1601, 1576, 1415, 1386, 1344, 1269, 1117, 1047, 1018, 871; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESIMS *m/z* 677.3266 $[M+Na]^+$ (calcd for $C_{37}H_{50}O_{10}Na$, 677.3296).

3.3.4. 1-O-Cinnamoyl-1-O-debenzoylohchinal (4)

White amorphous powder, $[\alpha]_D^{22}$: +23.0 (*c* 0.10, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}): 3391 (br), 2926, 2856, 1732, 1716, 1701, 1668, 1629, 1575, 1419, 1379, 1270, 1120, 1053, 1032, 872; ^1H and ^{13}C NMR data see Tables 1 and 2; HR-ESIMS *m/z* 637.2783 $[M+Na]^+$ (calcd for $C_{37}H_{42}O_8Na$, 637.2772).

3.3.5. Meliasenin S (5)

White amorphous powder, $[\alpha]_D^{22}$: −6.0 (*c* 0.1, $CHCl_3$); IR ν_{max}^{KBr} (cm^{-1}): 3464, 2925, 2854, 1649, 1459, 1383, 1256, 1155, 1129, 1089, 1057, 1022 cm^{-1}; ^1H and ^{13}C NMR data see Table 3; HR-ESIMS *m/z* 527.3655 $[M+Na]^+$ (calcd for $C_{31}H_{52}O_5Na$, 527.3707).

3.3.6. Meliasenin T (6)

White amorphous powder; IR ν_{max}^{KBr} (cm^{-1}): 3450, 2925, 2854, 1649, 1459, 1382, 1258, 1155, 1128, 1089, 1058, 1022 cm^{-1}; ^1H and ^{13}C NMR data see Table 3; HR-ESIMS *m/z* 527.3701 $[M+Na]^+$ (calcd for $C_{31}H_{52}O_5Na$, 527.3707).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2011.05.003.

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