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Minor Limonoids from *Melia toosendan* and their Antibacterial Activity

Abstract

In this study five new limonoids, toosendone [24,25,26,27-tetra-nor-6α-acetoxy-21,22-epoxy-7α-tigloyl-1α,3α,28-trihydroxya-potirucalla-(apoeupha)-14,20,22-trien-12-one, **1**] and 12-ethoxynimbolinins A – D (**2 – 5**), together with five known limonoids, 1-acetyltrichilinin (**6**), 1-cinnamoyltrichilinin (**7**), trichilinin B (**8**), 1,7-di-*O*-acetyl-14,15-deoxyhavanensin (**9**) and 12-*O*-methylnimbolinin B (**10**),were isolated from the fruits of *Melia toosendan*. Their structures and relative configurations were established based on spectroscopic analysis. Compound **4** exhibited significant antibacterial activity against the oral pathogen, *Porphyromonas gingivalis* ATCC 33277, with an MIC value of 15.6 μg/mL. Compounds **7** and **8** were also active against *P. gingivalis* ATCC 33277, with MIC values of 31.3 and 31.5 μg/mL respectively.

Key words
Melia toosendan · Meliaceae · limonoids · antibacterial · oral pathogen

Supporting information available online at
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Introduction

Melia toosendan is a wild plant growing mainly in the southwestern part of China. The fruits of *M. toosendan* have been used for the treatment of stomachache and hernia pain in traditional Chinese medicine. In early work, the chemical constituents of the fruits of *M. toosendan* have been studied extensively. Limonoids of different types including apo-euphols, meliacins, trichilinin, nimbolinin and others have been isolated. Limonoids from *Melia* species are attracting considerable interest because of their biological activities and variety of structures [1], [2], [3].

In the present work, five new limonoids (Fig. **1**), named toosendone (24,25,26,27-tetra-nor-6α-acetoxy-21,22-epoxy-7α-tigloyl-1α,3α,28-trihydroxy-apotirucalla-(apoeupha)-14,20,22-trien-12-one, **1**) and 12-ethoxynimbolinins A – D (**2 – 5**), together with five known limonoids, 1-acetyltrichilinin (**6**), 1-cinnamoyltrichilinin (**7**), trichilinin B (**8**), 1,7-di-*O*-acetyl-14,15-deoxyhavanensin (**9**) and 12-*O*-methylnimbolinin B (**10**), were isolated. In this paper, we report the isolation and structure elucidation of these new limonoids from the fruits of *M. toosendan*. The antibacterial activity of the isolated compounds against two oral pathogens (*Streptococcus mutans* ATCC 25175 and *Porphyromonas gingivalis* ATCC 33277) has been determined.

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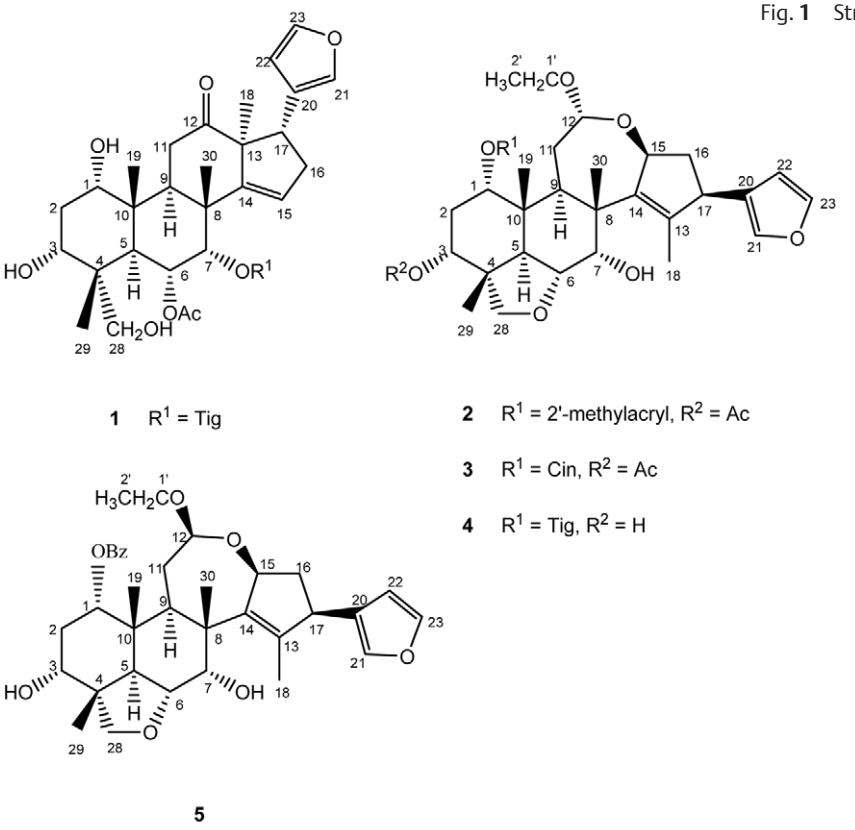

1 R^1 = Tig

2 R^1 = 2'-methylacryl, R^2 = Ac

3 R^1 = Cin, R^2 = Ac

4 R^1 = Tig, R^2 = H

5

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

Materials and Methods

General experimental procedures

Optimal rotations were taken in $CHCl_3$ on a Perkin-Elmer PE 241 polarimeter. The IR spectra were obtained on a Perkin-Elmer 16 PC FT-IR spectrophotometer. The 1D and 2D NMR spectra were run on a Bruker AV400 spectrometer, with TMS as an internal standard. The HR-ESI-MS were obtained on a PE Biosystems Mariner System 5140 LC/MS spectrometer. Column chromatography was carried out using silica gel (Merck; Darmstadt, Germany) and Sephadex LH-20 (Pharmacia; Uppsala, Sweden). HPLC was performed on a Waters Prep LC 4000 system with a UV detector and an X-bridge C-18 column (19 × 150 mm, 5 μm). TLC was performed on HPTLC plates (Merck), with compounds visualized by spraying with 4-(dimethylamino)benzaldehyde following by heating.

Plant material

The dried fruits of *Melia toosendan* were collected from Wanxian, Sichuan Province, People's Republic of China, in July 2005, and were identified by Prof. Qin Minjian (Department of Natural Medicinal Resources, China Pharmaceutical University, Nanjing, People's Republic of China). A voucher specimen (No. 24 – 88 – 53 – 3) was deposited in the herbarium of China Pharmaceutical University.

Extraction and isolation

The dried fruits of *M. toosendan* (20 kg) were crushed and extracted with ethyl acetate (40 × 1000 mL, 3 h each) under reflux three times. The solvent was evaporated under reduced pressure to obtain an extract (Fraction A, 300 g). Fraction A was subjected to silica gel column chromatography (13 × 85 cm, 230 – 400 mesh), eluted with a gradient of petroleum ether-EtOAc (9 : 1, 8 : 2, 7 : 3 and 6 : 4, each 18 000 mL) to afford fractions 1 – 20.

Fraction 9 (8 g) was chromatographed on silica gel (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of chloroform-acetone (100 : 1, 100 : 2 and 100 : 4, each 1500 mL) to afford fractions 9.1 – 9.5. Fraction 9.1 (3.8 g) was further chromatographed on silica gel (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of petroleum ether-acetone (90 : 10, 88 : 12, 85 : 15 and 80 : 20, each 1500 mL) to afford fractions 9.1.1 – 9.1.9. Fraction 9.1.6 (1.2 g) was chromatographed on Sephadex LH-20 [2 × 80 cm, $CHCl_3$-MeOH (1 : 1), 200 mL] to afford fractions 9.1.6.1 – 9.1.6.5. Fraction 9.1.6.4 (590 mg) was chromatographed on silica gel (2 × 33 cm, 230 – 400 mesh), eluting with $CHCl_3$ to afford fractions 9.1.6.4.1 – 9.1.6.4.5. Fraction 9.1.6.4.1 (25 mg) was purified by reversed phase preparative HPLC using a gradient of increasing acetonitrile (60 – 90%) in water at 18 mL/min for 20 min to give **9** (t_R = 10.3 min, 7 mg).

Fraction 11 (9 g) was chromatographed on silica gel (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of chloroform-acetone (100 : 1, 100 : 2 and 100 : 4, each 2000 mL) to afford fractions 11.1 – 11.9. Fraction 11.3 (1.7 g) was chromatographed on silica gel (3 × 39 cm, 230 – 400 mesh), eluting with a gradient of petroleum ether-acetone (80 : 20,75 : 25 and 70 : 30, each 600 mL) to afford fractions 11.3.1 – 11.3.10. Fractions 11.3.5 and 11.3.6 (43 mg) were purified by reversed phase preparative HPLC using a gradient of increasing acetonitrile (45 – 60%) in water at 18 mL/min for 20 min to afoord **10** (t_R = 11.4 min, 20 mg).

Fraction 12 (13 g) was chromatographed on silica gel (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of chloroform-acetone

(100 : 1, 100 : 3 and 100 : 5, each 2000 mL) to afford fractions 12.1 – 12.7. Fraction 12.2 (40 mg) was purified by reversed phase preparative HPLC using a gradient of increasing acetonitrile (55 – 65 %) in water at 18 mL/min for 20 min to give **2** (t_R = 11.2 min, 4 mg) and **3** (t_R = 14.7 min, 6 mg).

Fractions 13 and 14 (11 g) were chromatographed on silica gel (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of chloroform-acetone (100 : 2, 100 : 4, 100 : 6 and 100 : 8, each 2000 mL) to afford fractions 13.1 – 13.7. Fraction 13.2 (2.1 g) was purified by reversed phase preparative HPLC using a gradient of increasing acetonitrile (45 – 60 %) in water at 18 mL/min for 20 min to yield **7** (t_R = 16.1 min, 50 mg) and **8** (t_R = 13.5 min, 40 mg).

Fractions 15 and 16 (40 g) were chromatographed on silica gel (8 × 50 cm, 230 – 400 mesh), eluting with a gradient of chloroform-acetone (100 : 7, 100 : 10, 100 : 15, 100 : 30 and 100 : 50, each 8000 mL) to afford fractions 15.1 – 15.8. Fraction 15.4 (10 g) was chromatographed on silica gel (4 × 42 cm, 230 – 400 mesh), eluting with a gradient of petroleum ether-acetone (80 : 20, 75 : 25, 70 : 30 and 60 : 40, each 2000 mL) to afford fractions 15.4.1 – 15.4.7. Fraction 15.4.4 (700 mg) was chromatographed on silica gel (2 × 33 cm, 230 – 400 mesh), eluting with chloroform-acetone (100 : 8, 1000 mL) to afford fractions 15.4.4.1 – 15.4.4.5. Fraction 15.4.4.3 and 15.4.4.4 (150 mg) was purified by reversed phase preparative HPLC using a gradient of increasing acetonitrile (45 – 60 %) in water at 18 mL/min for 20 min to give **1** (t_R = 11.4 min, 6 mg), **5** (t_R = 13.1 min, 6 mg) and **6** (t_R = 8.9 min, 4 mg). Fraction 15.4.2 and 15.4.3 (450 mg) were chromatographed on silica gel (2 × 33 cm, 230 – 400 mesh), eluting with chloroform-acetone (100 : 8, 1000 mL) to afford fractions 15.4.2.1 – 15.4.2.6. Fraction 15.4.2.3 (30 mg) was purified by reversed phase preparative HPLC using a gradient of increasing acetonitrile (45 – 60 %) in water at 18 mL/min for 20 min to give **4** (t_R = 15.1 min, 5 mg).

Toosendone (**1**): amorphous powder; $[\alpha]_D^{25}$: + 15.8 (*c* 0.08, $CHCl_3$); IR (KBr): ν_{max} = 2928, 1709, 1258, 1029, 492 cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$), see Table **1S** (Supporting Information); HR-ESI-MS: *m/z* = 607.2880 $[M + Na]^+$ (calcd. for $C_{33}H_{44}O_9Na$: 607.2883).

12-Ethoxynimbolinin A (**2**): amorphous powder; $[\alpha]_D^{25}$: –29.0 (*c* 0.07, $CHCl_3$); IR (KBr): ν_{max} = 3444, 2928, 1718, 1244, 1053, 600 cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$), see Table **1S** (Supporting Information); HR-ESI-MS: *m/z* = 621.3044 $[M + Na]^+$ (calcd. for $C_{34}H_{46}O_9Na$: 621.3040).

12-Ethoxynimbolinin B (**3**): amorphous powder; $[\alpha]_D^{25}$: –44.4 (*c* 0.06, $CHCl_3$); IR (KBr): ν_{max} = 3412, 2927, 1712, 1245, 1054, 770 cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$), see Table **2S** (Supporting Information); HR-ESI-MS: *m/z* = 683.3198 $[M + Na]^+$ (calcd. for $C_{39}H_{48}O_9Na$: 683.3196).

12-Ethoxynimbolinin C (**4**): amorphous powder; $[\alpha]_D^{25}$: –43.2 (*c* 0.09, $CHCl_3$); IR (KBr): ν_{max} = 3435, 2927, 1706, 1262, 1065, 755 cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$), see Table **2S** (Supporting Information); HR-ESI-MS: *m/z* = 593.3094 $[M + Na]^+$ (calcd. for $C_{33}H_{46}O_8Na$: 593.3090).

12-Ethoxynimbolinin D (**5**): amorphous powder; $[\alpha]_D^{25}$: –6.1 (*c* 0.08, $CHCl_3$); IR (KBr): ν_{max} = 3446, 2929, 1716, 1271, 1071, 771 cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$), see Table **3S** (Supporting Information); HR-ESI-MS: *m/z* = 615.2932 $[M + Na]^+$ (calcd. for $C_{35}H_{44}O_8Na$: 615.2934).

MIC determinations

The MIC values of compounds **4, 7** and **8** against selected oral bacteria (ATCC; Manassas, VA, USA) were determined using liquid cultures in 96-well culture plates according to a modification of the method described by Shapiro et al. [4] 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, USA) 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 ($OD_{630} \leq 0.05$) was observed was defined as the minimum inhibitory concentration (MIC).

Supporting information

^1H-NMR, ^{13}C-NMR and 2D NMR data for compounds **1 – 5** are available as Supporting Information.

Results and Discussion

The dried fruits of *M. toosendan* were extracted with ethyl acetate. The concentrated extract was separated using column chromatography on silica gel in addition to low-pressure chromatography and Sephadex LH-20, and further purified by reversed-phase preparative HPLC to afford five new limonoids (**1 – 5**) and five known limonoids (**6 – 10**).

The known compounds 1-acetyltrichilinin (**6**) [5], 1-cinnamoyltrichilinin (**7**) [5], trichilinin B (**8**) [6], 1,7-di-*O*-acetyl-14,15-deoxyhavanensin (**9**) [7] and 12-*O*-methylnimbolinin B (**10**) [8], were identified by comparison of their physical properties and spectroscopic data with those described in the literature.

Compoud **1** was obtained as an amorphous powder, and its molecular formula $C_{33}H_{44}O_9$ was established by HR-ESI-MS. The IR spectrum showed ν = 2928 cm^{-1} (hydroxy group) and 1709 cm^{-1} (carbonyl group) absorption peaks. The combined analysis of its ^1H- and ^{13}C-NMR spectra (Table **1**) revealed the presence of a tigloyl group [δ_H = 6.92 (1H), 1.81 (3H), 1.86 (3H), δ_C = 166.9 (CO), 138.4, 128.2, 14.6, 12.2] and an acetyl group [δ_H = 2.02, (δ_C = 21.3), δ_C = 169.2]. According to the ^{13}C-NMR and HSQC spectra, the remaining signals consisted of 26 carbons: four methyls, four methylenes, eleven methines, six quaternary carbons and one carbonyl (δ_C = 213.3). A furan ring [δ_H = 6.49 (1H), 7.26 (1H), 7.30 (1H), δ_C = 124.6, 112.4, 140.7, 142.4] was obviously ob-

Table **1** ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100MHz) data of compounds **1 – 5** in $CDCl_3$ (δ values; *J* values in parentheses)

Position	*1*		*2*		*3*		*4*		*5*	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	3.45 m	71.4	4.69 m	71.4	4.80 m	71.3	4.93 m	72.8	5.24 m	73.6
2a 2b	2.29 m 2.10 m	28.8	2.20 m	27.3	2.27 m	27.5	2.06 m 2.25 m	30.0	2.38 m 2.11 m	30.9
3	3.81 s	80.4	4.92 t	71.6	4.94 s	71.6	3.81 d (9.2)	71.2	3.90 s	70.7
4		40.3		42.8		42.8		44.3		44.1
5	3.06 d (2.0)	32.6	2.89 d (12.6)	38.2	2.97 d (12.6)	38.4	2.80 d (12.4)	37.2	2.92 d (12.5)	37.4
6	5.63 d (2.3)	70.6	4.02 dd (12.6, 2.8)	73.9	4.08 dd (12.6, 2.9)	73.9	4.07 dd (12.4,2.7)	73.8	4.09 dd (12.5, 2.6)	73.8
7	5.60 m	74.9	4.37 d (2.8)	73.3	4.43 d (2.8)	73.5	4.41 d (2.7)	73.4	4.42 d (2.6)	73.3
8		42.6		46.2		46.2		46.1		46.3
9	3.56 m	37.6	3.16 d (10.4)	34.6	3.25 d (10.3)	34.8	3.08 d (10.4)	34.9	2.72 d (9.7)	37.3
10		43.5		40.8		40.7		41.1		41.7
11a 11b	2.68 m 2.27 m	34.9	1.79 m 1.53 m	32.1	1.78 m 1.58 m	32.1	1.81 m 1.52 m	32.1	1.63 m	33.1
12		213.3	4.74 m	96.5	4.75 br s	96.5	4.74 br s	96.3	3.92 br s	103.5
13		61.4		138.7		138.7		138.3		138.9
14		154.5		144.6		144.6		144.6		143.6
15	5.52 s	122.7	5.03 d (8.0)	77.3	5.10 d (7.8)	77.2	5.02 d (7.8)	77.2	4.27 d (7.9)	81.7
16a 16b	2.41 m 1.55 m	33.9	2.55 m 1.57 m	38.0	2.54 m 1.58 m	38.0	2.56 m 1.57 m	38.0	2.45 m 1.52 m	37.8
17	3.45 m	42.5	3.43 m	46.6	3.45 d (9.8)	46.7	3.40 m	46.6	3.37 d (8.6)	46.2
18	1.03 s	18.7	1.72 s	16.0	1.76 s	16.1	1.74 s	16.1	1.71 s	16.1
19	1.03 s	17.6	0.95 s	15.7	0.99 s	15.9	0.96 s	15.9	1.02 s	16.2
20		124.6		128.8		128.8		128.8		128.6
21	7.26 s	140.7	7.25 s	139.0	7.26 s	139.0	7.27 s	138.9	7.24 s	138.9
22	6.49 s	112.4	6.39 s	110.4	6.41 s	110.5	6.40 s	110.4	6.42 s	110.5
23	7.30 s	142.4	7.29 s	142.8	7.30 s	142.8	7.31 s	142.8	7.26 s	142.8
28a 28b	3.67m 3.42 m	73.8	3.57 br s	78.1	3.63 m	78.0	4.10 d (7.4) 3.62 d (7.5),	78.2	4.15 d (7.4) 3.65 d (7.4),	78.1
29	0.76 s	17.1	1.18 s	19.7	1.21 s	19.7	1.14 s	20.1	1.16 s	19.9
30	1.20 s	25.9	1.33 s	20.7	1.37 s	20.8	1.34 s	20.7	1.36 s	20.8
12-O-ethyl										
1′			3.43 m	62.4	3.37 m	62.3	3.40 m	62.3	3.18 m, 3.59 m	63.6
2′			1.01 t (7.0)	14.9	0.94 t (7.0)	15.1	0.99 t (7.1)	14.8	1.05 t (7.0)	14.9
OAc Ac-CO Ac-Me	 2.02 s	 169.2 21.3	 1.92 s	 170.3 20.8	 1.88 s	 170.3 21.1				
$OCOC(CH_3)CH_2$										
1′				165.9						
2′				137.4						
3′			5.54 d (2.0), 6.22 d (2.0)	125.3						
2′-CH_3			2.03 s	18.4						
OTig										
1′		166.9						165.9		
2′		128.2						129.0		
3′	6.92 dd (1.2,7.1)	138.4					6.94 dd (1.2,7.0)	137.6		
2î-CH_3	1.86 s	12.2					1.90 s	12.3		

Table **1** Cont.

Position	1		2		3		4		5	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
3′-CH_3	1.81 d (7.1)	14.6					1.81 d (7.0)	14.5		
OCin										
1′						165.7				
2′					6.49 d (16.0)	119.7				
3′					7.76 d (16.0)	144.1				
4′						134.7				
5′, 9′					7.52 m	127.9				
6′, 8′					7.39 m	129.0				
7′					7.39 m	130.2				
OBz										
1′										164.6
2′										129.8
3′, 7′									8.11 d (7.2)	129.6
4′, 6′									7.50 t	129.0
5′									7.62 t	133.6

served from the NMR spectra. From the above data, accordingly, a meliacin-type limonoid derivative was suggested [2]. The HMBC spectrum of compound **1** (Table **1 S**, Supporting Information) showed the $^2J_{H,C}$-connectivities of H-11 and C-12 (δ_C = 213.3), as well as the $^3J_{H,C}$-connectivities of CH_3 – 18/C-12, indicating that the carbonyl group should be placed at C-12. The $^3J_{CH}$ correlation between H-7 (δ_H = 5.60) and the tigloate carbonyl group (δ_C = 166.9) indicated that the tigloyl group was situated at C-7. The HMBC correlation between H-6 (δ_H = 5.63) and the acetate carbonyl group (δ_C = 169.2) demonstrated that the acetyl group could be at the C-6 position. Three hydroxy groups were placed at C-1, C-3 and C-28, respectively. The stereochemistry of compound **1** was determined by a NOESY experiment (Table **1S**, Supporting Information). The NOE correlations of CH_3 – 29/H-3, CH_3 – 29/H-6, and CH_3 – 29/CH_3 – 19 suggested the β-orientation of H-3 and thus the β-orientations of CH_3 – 29, H-6 and CH_3 – 19. The NOE correlation between CH_3 – 19 and H-1 revealed that H-1 was β-oriented. H-7 showed NOE correlations with CH_3 – 30 and CH_3 – 19, indicating that H-7 was also in the β configuration. Observation of NOE effects at H-9/H-5 and H-9/CH_3 – 18 indicated the α-configurations of H-9, H-5 and CH_3 – 18. Thus, the structure of compound **1** was established as 24,25,26,27-tetra-nor-6α-acetoxy-21,22-epoxy-7α-tigloyl-1α,3α,28-trihydroxyapotirucalla-(apoeupha)-14,20,22-trien-12-one.

Compound **2**, isolated as an amorphous powder, had a molecular formula of $C_{34}H_{46}O_9$ deduced from HR-ESI-MS. The IR spectrum showed absorption peaks at ν = 3444 cm^{-1} (hydroxy group) and 1718 cm^{-1} (carbonyl group). The ^{1}H- and ^{13}C-NMR signals (Table **1**) of **2** were assigned by different 2D NMR experiments. The combined analysis of its ^{1}H, ^{13}C and 2D NMR spectra revealed the presence of an acetyl group [δ_H = 1.92 (δ_C = 20.8), δ_C = 170.3] and the 2-methylacryl group COC(CH_3) = CH_2 [δ_H = 5.54 (1H, d, J = 2Hz), 6.22 (1H, d, J = 2Hz), 2.03 (3H, s), δ_C = 165.9 (CO), 137.4,125.3,18.4]. The ^{1}H-^{1}H COSY spectrum indicated the presence of an ethoxyl group [δ_H = 1.01 (3H, t, J = 7.0Hz), δ_C = 14.9, δ_H 3.43 (2H, m), 62.4]. In the HMBC spectrum (Table **1 S**, Supporting Information), the $^2J_{CH}$ correlation between the ethoxylic methyl (δ_H = 1.01) and the ethoxylic methylene (δ_C = 62.4) further confirmed the existence of the ethoxy group. According to the ^{13}C-NMR and HSQC spectra, the remaining signals consisted of 26 carbons: four methyls, four methylenes, twelve methines and six quaternary carbons. A furan ring [δ_H = 6.39 (1H), 7.25 (1H), 7.29 (1H), δ_C = 128.8, 110.4, 139.0, 142.8] was also apparent from the NMR spectra. Further comparison of the chemical shifts with those of limonoids isolated from *M. toosendan* indicated that a ring C-seco-nimbolinin skeleton was present [2], [3], [9], [10]. The chemical shifts of H-1, H-3, H-7 and H-12 were assigned as 4.69, 4.92, 4.37 and 4.74, respectively, based on the HSQC and HMBC spectra. In the HMBC spectrum (Table **1 S**, Supporting Information), the $^3J_{CH}$ correlation between H-1 (δ_H = 4.69) and the carbonyl (δ_C = 165.9) of the 2-methylacryl group indicated that the 2-methylacryl group was situated at C-1. The $^3J_{CH}$ correlation between H-3 (δ_H = 4.92) and the carbonyl (δ_C = 170.3) of acetyl group revealed the acetyl group was at C-3 position. The $^3J_{CH}$ correlation between H-12 (δ_H = 4.74) and the ethoxylic methylene (δ_C = 62.4) demonstrated that the ethoxy group was placed at C-12. The hydroxy group was located at C-7 (δ_C = 73.3). The stereochemistry of **2** was determined by a NOESY experiment (Table **1 S**, Supporting Information). The NOE correlations of CH_3 – 29/H-3, CH_3 – 29/H-6 and CH_3 – 29/CH_3 – 19 suggested the β-orientation of H-3 and thus the β-orientations of CH_3 – 29, H-6 and CH_3 – 19. The NOE correlation of CH_3 – 19/H-1 revealed that H-1 was in the β-configuration. In turn, H_b-11 (δ_H = 1.53, β-orientation) had a NOE correlation with H-12, which indicated that H-12 was in the β-configuration. The NOE correlations between H-7/H-6 and H-7/CH_3 – 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 an α configuration for H-17. In conclusion, the structure of compound **2** was established as 1α,2′-methylacryl-3α-acetyl-12α-ethoxy-7α-hydroxynimbolinin.

Compound **3**, amorphous powder, was assigned the molecular formula $C_{39}H_{48}O_9$ based on its HR-ESI-MS. The ^{1}H-NMR and ^{13}C-NMR data of **3** (Table **1**) demonstrated that compound **3** consisted of a cinnamoyl group [δ_H = 6.49 (1H), 7.76 (1H), 7.52 (2H), 7.39 (2H), 7.39 (1H), δ_C = 165.7 (CO), 119.7, 144.1, 134.7, 127.9, 129.0, 130.2], an acetyl group [δ_H = 1.88 (δ_C = 21.1), δ_C = 170.3], an ethoxy group [δ_H = 0.94 (3H, t, J = 7.0Hz), δ_C = 15.1, δ_H = 3.37 (2H, m), δ_C = 62.3] and a hydroxy group. By comparison the remaining NMR data with compound **2**, the same carbon skeleton was assigned. The presence of the cinnamoyl group at the C-1 position was confirmed by the observation of the $^3J_{CH}$ connectivities in the HMBC spectrum (Table **2 S**, Supporting Information) between H-1 (δ_H = 4.80) and the carbonyl of the cinnamoyl group (δ_C = 165.7). In addition, according to the HMBC spectrum, the acetyl group was placed at C-3, the hydroxy group was attached to C-7 and the ethoxyl group was situated at C-12. The stereochemistry of **3** was established by a NOESY experiment (Table **2 S**, Supporting Information). Thus, the structure of compound **3** was elucidated as 1α-cinnamoyl-3α-acetyl-12α-ethoxy-7α-hydroxynimbolinin.

Compound **4** was obtained as an amorphous powder, and its molecular formula was determined as $C_{33}H_{46}O_8$ by HR-ESI-MS. The ^{1}H-NMR and ^{13}C-NMR spectra (Table **1**) showed the presence of a tigloyl group [δ_H = 6.94 (1H), 1.81 (3H), 1.90 (3H), δ_C = 165.9 (CO), 137.6, 129.0, 14.5, 12.3] and an ethoxy group [δ_H = 0.99 (3H, t, J = 7.0Hz), δ_C = 14.8, δ_H = 3.40 (2H, m), δ_C = 62.3]. On the basis of the NMR data, the same carbon skeleton as compounds **2** and **3** was proposed. According to the HMBC spectrum (Table **2S**, Supporting Information), the tigloyl group was placed at C-1 and the two hydroxy groups were linked to C-3 (δ_C = 71.2) and C-7 (δ_C = 73.4) respectively. The relative configuration of **4** was established by a NOESY experiment (Table **2 S**, Supporting Information). Therefore, the structure of compound **4** was established as 3α,7α-dihydroxy-12α-ethoxy-1α-tigloylnimbolinin.

Compound **5** was obtained as an amorphous powder, and its molecular formula $C_{35}H_{44}O_8$ was established by HR-ESI-MS. According to the NMR data (Table **1**), the structure of **5** was found to consist of a benzoyl group [δ_H = 8.11 (2H), 7.50 (2H), 7.62 (1H), δ_C = 164.6 (CO), 129.8, 129.6, 129.0, 133.6] and an ethoxy group [δ_H = 1.05 (3H, t, J = 7 Hz), δ_C = 14.9, δ_H = 3.18 (1H, m), 3.59 (1H, m), δ_C = 63.6]. From the NMR data, the carbon skeleton of compound **5** was assigned as the same as those of compounds **2–4.** The benzoyl group was linked to C-1 according to the HMBC spectrum (Table **3 S**, Supporting Information). The stereochemistry of compound **5** was established by a NOESY experiment (Table **3 S**, Supporting Information). 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 = 103.5). Thus, the structure of compound **5** was characterized as 1α-benzoyl-3α,7α-dihydroxy-12β-ethoxy-nimbolinin.

All the compounds were tested for their antibacterial activity against oral pathogens *Streptococcus mutans* ATCC 25175 and *Porphyromonas gingivalis* ATCC 33277. Compound **4** exhibited significant antibacterial activity against the oral pathogen, *P. gingivalis* ATCC 33277, with an MIC value of 15.6 μg/mL. Compounds **7** and **8** were also active against *P. gingivalis* ATCC 33277, with MIC values of 31.3 and 31.5 μg/mL respectively. All the compounds showed no activity against *S. mutans* ATCC 25175.

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