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Two new nimbolinin- and trichilin-class limonoids isolated from the fruits of *Melia azedarach*

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[ABSTRACT] Two new furan fragment isomerized limonoids, meliazedalides A and B (compounds **1** and **2**), were isolated from the fruits of *Melia azedarach* Linn.. Their chemical structures were elucidated on the basis of HR-ESI-MS and 1D and 2D NMR data, which belonged to nimbolinin- and trichilin-class, respectively. Compound **2** exhibited weak inhibitory effect on NO production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages with IC_{50} being 37.41 μmol·L^{-1}.

[KEY WORDS] *Melia azedarach*; Meliaceae; Limonoid; Anti-inflammatory

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Introduction

Melia azedarach Linn., a timber of Meliaceae family, is distributed in Sichuan, Yunnan, Guizhou and Jiangsu Provinces of China. Its stem and barks are recorded in the Chinese Pharmacopoeia and have been used in traditional Chinese medicine for acesodyne and anthelmintic treatments [1]. Its fruits are used as alternative of toosendan fruits in some regions of China, which are another famous anthelmintic and analgesic TCMs from *M. toosendan* in some areas of China [1-2]. Limonoids and tirucallane-type triterpenoids are the main constituents of this plant [3-6], which have been reported to possess a variety of biological properties, such as antibacterial, cytotoxic, anti-inflammatory, and antifeedant activities [7-10]. Toosendan fruits have attracted many attentions of researchers [11-13], but the azedarach fruits are largely ignored. Therefore, we carried out this project to demonstrate the limonoids constituents of azedarach fruits. As a result, two new limonoids (compounds **1** and **2**) with isomerized furan fragment (Fig. 1) were isolated and identified from the fruits of *M. azedarach*. Their chemical structures were identified by spectroscopic methods, including HR-ESI-MS and 1D- and 2D-NMR. Herein, we report the isolation, structure elucidation, and bioactivities of these two new limonoids.

Results and Discussion

Meliazedalide A (**1**) was obtained as a white amorphous powder with a molecular formula of $C_{33}H_{44}O_{11}$ (HR-ESI-MS ion at *m/z* 639.2778 $[M + Na]^+$, Calcd. for $C_{33}H_{44}NaO_{11}$ 639.2776), which indicated 12 degrees of unsaturation. The ^1H NMR spectrum exhibited four angular methyl groups (δ_H 1.89, 1.48, 1.15, and 0.94), one acetyl group (δ_H 2.05), and one *Tig*-group (δ_H 6.88, 1.87, and 1.80). The ^{13}C NMR spectrum showed the existence of three ester group (δ_C 171.2, 170.3, and 166.4) and two double bonds (δ_C 146.2, 140.4, 137.4, and 128.7). The characterized proton singlets at δ_H 5.99 and 5.85, carbon resonances at δ_C 171.2, 118.6, and 98.2 indicated the presence of 21-hydroxybutenolide moiety in structure of **1** by comparing the NMR data of entangolensins F [14] and khaysenelide J [15]. The aforementioned data as well as other key information from 2D NMR studies (HMBC, HSQC, and ROESY) suggested that **1** afforded a structure closely related to that of 1-deacetylnimbolinin B [16], with the only difference in the replacement of the furan ring by 21-hydroxybutenolide moiety at C-17, which was deduced from the HMBC correlations (Fig. 2) from H-21 to C-17, C-22, and C-23. Thus, the planar structure of **1** was determined. The ROESY correla-

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tions (Fig. 2) from H-28a to H-5, H-5 to H-9, and H-9 to H-15 indicated that these protons were *α*-oriented. Consequently, ROESY correlations from Me-19 to H-1, Me-19 to H-11b, Me-19 to Me-29, Me-19 to Me-30, Me-29 to H-3, Me-29 to H-6, H-11b to H-12, H-6 to H-7, Me-18 to H-7, and Me-18 to H-17 revealed that they were assigned as *β*-orientation. Therefore, the relative configuration of meliazedalide A (**1**) was determined as shown in Fig. 1.

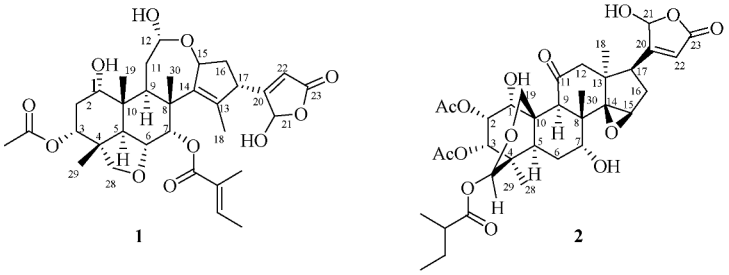

Fig. 1 Structures of compounds 1 and 2

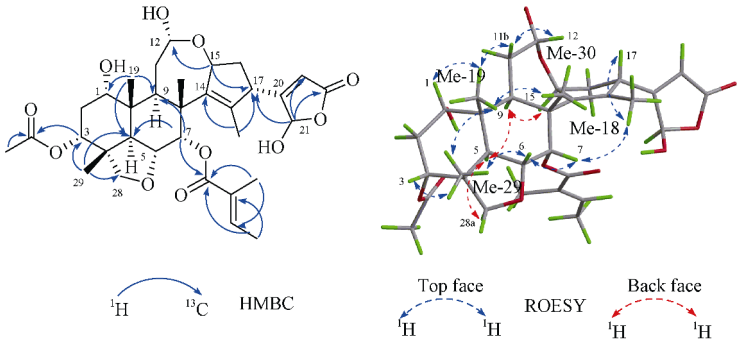

Fig. 2 Key HMBC and ROESY correlations of compound 1

Meliazedalide B (**2**) was obtained as a white amorphous powder with a molecular formula of $C_{35}H_{46}O_{12}$ (HR-ESI-MS ion at *m/z* 713.2779 $[M + Na]^+$, Calcd. for $C_{35}H_{46}NaO_{12}$ 713.2780), which indicated 13 degrees of unsaturation. The whole features of ^1H and ^{13}C NMR spectrum of **2**, especially of the signal of acetal carbon at 93.6 and two harmonic coupled oxygenated methylene signals at δ_H 4.36 and 4.45 (each d, J = 13.5, 13.0 Hz), suggested that this compound should be a trichilin-type limonoids with katal C-29 and C29-O-C19 bridge as toosendanin [17] from Melia plants. The NMR data of **2** revealed the presence of 21-hydroxybutenolide moiety, by the characterized NMR data of the proton singlets at δ_H 5.90 and 5.85 and carbon resonances at δ_C 170.2, 168.8, 120.3, and 98.3, same as those of compound **1**. The aforementioned data as well as other key information from 2D NMR studies (HMBC, HSQC and ROESY) suggested that **2** afforded a structure closely related to that of trichilin D [17], with the only difference in the replacement of the furan ring by 21-hydroxybutenolide moiety at C-17, which was deduced from the HMBC correlations (Fig. 3) from H-22 to C-21, C-23. Thus, the planar structure of **2** was determined. The ROESY correlations (Fig. 3) from Me-18 to H-9, Me-18 to H-15, and H-9 to H-17 indicated that these protons were *α*-oriented. Consequently, ROESY correlations from H-19 to H-1, H-1 to H-2, H-2 to H-3, Me-30 to H-19, and Me-30 to H-7 revealed that they were assigned as *β*-orientation. Therefore, the relative configuration of meliazedalide B (**2**) was established as shown in Fig. 1.

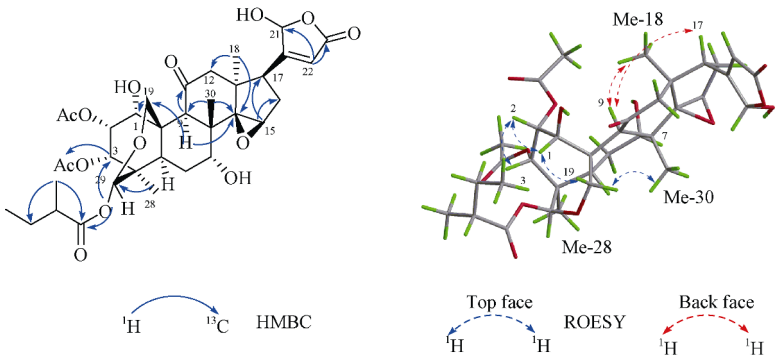

Fig. 3 Key HMBC and ROESY correlations of compound 2

Compounds **1** and **2** were evaluated for their inhibitory effects on NO production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages with L-NMMA as the positive control. The results revealed that compound **2** exhibited weak NO inhibitory activities, with IC_{50} being 37.41 μmol·L^{-1}. These two new limonoids with isomerized furan fragment showing inhibitory effects on NO production provided further evidence for our previous finding that the isomerized furan fragment played important roles in the bioactivities of limonoids.

Experimental

General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan). IR spectra were recorded on Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany). UV spectra were recorded on Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). NMR spectra were acquired at 500 MHz (^{1}H) and 125 MHz (^{13}C) on a Bruker Avance III NMR spectrometer (Bruker, Karlsruhe, Germany) with tetramethylsilane as internal standard. HR-ESI-MS analyses were carried out on an Agilent 6520 B UPLC-Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Silica gel (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), MCI gel (Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 (Pharmacia) and RP-C_{18} column (40–63 μm, FuJi, Japan) were used for column chromatography. Semi-preparative HPLC was performed on a Shimadzu LC-20AR instrument with a SPD-20A detector using a shimpack ODS column (20 mm × 250 mm, 15 μm). Analytical HPLC was carried out on an Agilent 1100 Series instrument with a DAD detector using a BDS HYPERSIL column (150 mm × 4.6 mm, 5 μm). All the solvents used were analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd., Huaian, China).

Plant materials

The fresh fruits of *M. azedarach* were collected from the Jiangning campus of China Pharmaceutical University, Nanjing, Jiangsu Province, China, in November 2016, and were authenticated by Prof ZHANG Mian, the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 2016-MMA) was deposited in the Testing & Analysis Center, Nanjing Normal University, Nanjing, China.

Extraction and isolation

The fresh fruits of *M. azedarach* (16.0 kg) were extracted by ultrasounding with 95% ethanol thrice, 4 h each. The EtOH extract was concentrated under reduced pressure to obtain a residue (875 g), which was suspended in H_2O and partitioned with CH_2Cl_2. The CH_2Cl_2 fraction (183.01 g) was subjected to a silica gel column eluted with CH_2Cl_2–MeOH in a gradient (100 : 0 to 0 : 100, *V*/*V*) to afford six fractions (Frs. A–F) after TLC and HPLC analysis. Fr. E (7.99 g) was run on a MCI column using a step gradient of MeOH–H_2O (20 : 80 to 100 : 0) to afford six sub-fractions (E1–6). Separation of Fraction E3 (578.6 mg) *via* Sephadex LH-20 gel columns afforded six sub-fractions (Frs. E3a–3f). Fr. E3b (491.5 mg) was repeatedly separated by semi-preparative HPLC using MeOH–H_2O (55 : 45, *V*/*V*, 6 mL·min^{-1}) and MeCN–H_2O (37 : 63, *V*/*V*, 8 mL·min^{-1}) as the mobile phase to give compound **1** (3.1 mg). Fr. E4 (1.45 g) was run on a ODS column using a step gradient of MeOH–H_2O (50 : 50, 63 : 37, 80 : 20, 100 : 0) to afford eight sub-fractions (Frs. E4a–4h). Fr. E4d (142 mg) was repeatedly separated by semi-preparative HPLC using MeCN–H_2O (40 : 60, *V*/*V*, 9 mL·min^{-1}) as the mobile phase to give compound **2** (2.7 mg).

Meliazedalide A (**1**): White, amorphous power, $[\alpha]_D^{25}$ −7.2 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.14) nm; IR (KBr) ν_{max}: 2930, 1736, 1378, 1258, 1134, 1079, 1046, 953, 730 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HR-ESI-MS *m/z* 639.2778 $[M + Na]^+$ (Calcd. for $C_{33}H_{44}NaO_{11}$, 639.2776).

Table 1 ^{1}H NMR (500 Hz) and ^{13}C NMR (125 MHz) spectroscopic data for compounds 1 and 2 in $CDCl_3$

No.	1		2	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	71.3	3.65, s	71.8	4.22, d (4.5)
2a	29.5	2.17, m	68.6	5.91, d (4.5)
2b		2.26, m		
3	72.4	4.98, t (2.5)	73.2	5.52, d (4.5)
4	42.5		41.1	
5	39.4	2.71, d (12.7)	40.9	2.51, dd (14.0, 7.0)
6a	72.5	4.10, dd (12.5, 3.0)	26.7	1.57, m
6b				2.00, m
7	74.5	5.71, d (2.8)	70.8	3.70, m
8	45.4		43.3	
9	36.2	3.07, d (7.5)	48.6	4.52, s
10	41.0		42.2	
11a	31.1	1.65, m	211.1	
11b		1.77, m		
12a	91.8	5.28, s	48.9	2.48, s
12b				2.57, s
13	140.4		42.7	
14	146.2		72.6	
15	77.9	5.18, d (7.35)	57.4	3.71, s
16a	35.9	1.77, m	31.6	1.94, dd (13.0, 11.0)
16b		2.18, m		2.30, dd (13.0, 6.0)
17	49.6	3.32, m	42.1	2.75, m
18	16.5	1.89, s	21.4	1.36, s
19a	16.7	0.94, s	64.4	4.36, d (13.5)
19b				4.45, d (13.0)
20	–[a]		168.8	
21	98.2	5.99, s	98.3	5.90, s
22	118.6	5.85, s	120.3	5.85, s

Continued

No.	1		2	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
23	171.2		170.2	
28a	78.1	3.44, d (7.65)	19.0	0.84, s
28b		3.52, d (7.65)		
29	18.8	1.15, s	93.6	5.76, s
30	20.8	1.48, s	21.8	1.06, s
2-OAc			169.2	
			21.1	2.03, s
3-OAc	170.3		170.7	
	21.2	2.05, s	20.9	2.14, s
1'	166.6		175.6	
2'	128.7		28.2	2.74, m
3'a	137.4	6.88, qd (7.0, 1.5)	26.3	1.72, m
3'b				2.07, m
4'	14.7	1.80, d (7.0)	11.5	0.94, t (7.5)
5'	12.4	1.87, s	16.5	1.19, d (7.0)

[a] Signal cannot be observed clearly from 1D- and 2D-NMR

Meliazedalide B (**2**): White, amorphous power, $[\alpha]_D^{25}$ −0.4 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.75) nm; IR (KBr) ν_{max}: 2921, 2851, 1745, 1648, 1468, 1382, 1255, 1063, 958 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HR-ESI-MS *m/z* 713.2779 $[M + Na]^+$ (Calcd. for $C_{35}H_{46}NaO_{12}$, 713.2780).

Nitric oxide (NO) inhibitory assay

Compounds **1** and **2** were evaluated for their inhibitory effects on NO production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages as described in the literatures [18]. Mouse macrophage cell line (RAW 264.7) was obtained from the Chinese Academy of Science Cell Bank (Shanghai, China).

Cells were cultured in DMEM with 10% FBS, penicillin (100 U·mL^{-1}), and streptomycin (100 μg·mL^{-1}) in a humidified atmosphere with 5% CO_2 at 37 °C. NO production was determined by the level of accumulated nitrite in cell culture supernatants using the Griess reagent from Beyotime Institute of Biotechnology (Jiangsu, China). Briefly, RAW264.7 cells (8×10^5 cells/mL) were incubated in 96-well plates and pretreated with test samples for 1 h, following by incubation with 1 μg·mL^{-1} LPS stimulation for 18 h. Then, the culture supernatant was mixed with an equal volume of Griess reagent. The absorbance of the mixture was read at 540 nm using a microplate reader. All the experiments were conducted for three independent replicates, with L-NMMA as a positive control, which was obtained from the Beyotime Institute of Biotechnology (Jiangsu, China).

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