Tetrahedron 72 (2016) 7481–7487

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Limonoids with modified furan rings from root barks of *Toona sinensis*

Jun-He Li [a], Yi Li [b], Fa-Liang An [a], Miao-Miao Zhou [a], Jie Luo [a], Kai-Li Jian [a], Jun Luo [a,*], Ling-Yi Kong [a,*]

[a] State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People's Republic of China
[b] Testing & Analysis Center, Nanjing Normal University, Nanjing 210046, People's Republic of China

ARTICLE INFO

Article history:
Received 17 August 2016
Received in revised form 23 September 2016
Accepted 27 September 2016
Available online 28 September 2016

Keywords:
Toona sinensis (A. Juss.) Roem.
Limonoids
Modified furan rings
Cytotoxicity
anti-Inflammatory activity

ABSTRACT

Toonasinemines A–K (**1**–**12**), 12 new azadirone- and gedunin-type limonoids with eight-classes of modified furan rings, were isolated from the root barks of *Toona sinensis* (A. Juss.) Roem. Their structures were elucidated based on detailed spectroscopic analyses, and the absolute configurations of **1**–**9** were established by the electronic circular dichroism exciton chirality method. The lactam-bearing moieties in compounds **1**–**7** were rare in limonoids family. Results of bioactivities screening exhibited that **1**, **4**, and **8** showed moderate cytotoxicities against HepG2, MCF-7, and U2OS human cancer cell lines, and **1**, **2**, **6**, **8**, and **9** exhibited marked inhibitory effects on nitric oxide (NO) production in LPS-activated RAW 264.7 macrophages at nontoxic concentration.

1. Introduction

Toona sinensis (A. Juss.) Roem. (Meliaceae), a perennial deciduous arbour, is widely distributed and cultivated in eastern and southeastern Asia, and it has more than 2000 years of cultivation history in China. The leaves of *T. sinensis*, which contain a distinct flavour, have been the vegetarian cuisine and also used as a traditional Chinese herbal medicine for treatment of itch, dysentery, and enteritis, while the bark was used as astringent and depurative substance.[1–3] In the previous investigations of *T. sinensis*, a variety of ring A-*seco* limonoids and ring B-*seco* limonoids were reported as its main constituents.[4–6] During an ongoing search for limonoids with novel structure and significant biological activities, the root barks of *T. sinensis* were chosen as our research subject. As a result, 12 new azadirone- and gedunin-type limonoids with diverse modified furan rings, Toonasinemines A–K (**1**–**12**), were isolated from the root barks of *T. sinensis*. Their structures were elucidated on the basis of spectroscopic methods including high resolution-electrospray ionization (HR-ESI)-MS, one and two dimensional (1D and 2D)-NMR. The N-containing modification in furan rings of compounds **1**–**7**, known as limonoid-based alkaloids, were rare in limonoids family.[7–14] Due to the structure of the rare modified furan rings, all new limonoids were evaluated in vitro for their potential biological activities, such as cytotoxicity and anti-inflammatory activity. Herein, we describe the isolation and structural elucidation of these new limonoids as well as their bioactivities.

2. Results and discussion

Toonasinemine A (**1**) was obtained as a white, amorphous powder. Its molecular formula was determined to be $C_{28}H_{35}NO_6$ from the $[M+H]^+$ peak at *m*/*z* 482.2536 (calcd 482.2537). The ^1H NMR data of **1** (Table 1) displayed resonances characteristic of five tertiary methyl groups (δ_H 1.06, 1.07, 1.07, 1.20 and 1.22, each 3H, s) and one acetate methyl group (δ_H 2.02, 3H, s). The ^{13}C NMR (Table 1) spectrum indicated the presence of six methyls, four methylenes, eight methines, and 10 quaternary carbons with the help of HSQC data. ^1H and ^{13}C NMR data combined with 2D NMR spectra suggested **1** as being a azadirone-type limonoid with intact A–D rings. The ^{13}C NMR signals of C-1 (δ_C 157.7), C-2 (δ_C 125.9), and C-3 (δ_C 204.3) and the ^1H NMR signals of a pair of AB doublets at δ_H 5.87 and 7.16 (J=10.5 Hz) suggested that the A-ring of **1** possessed a 1-en-3-one system. The signals of C-14 (δ_C 72.4), C-15 (δ_C 57.4), and H-15 (δ_H 3.92) indicated the presence of 14,15-epoxide. In HMBC spectrum, the correlations (Fig. 2) from H-15 (δ_H 3.39, s)/H-

* Corresponding authors. Fax: +86 25 8327 1405; e-mail addresses: luojun1981ly@163.com (J. Luo), cpu_lykong@126.com (L.-Y. Kong).

http://dx.doi.org/10.1016/j.tet.2016.09.061
0040-4020/

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of compounds **1**–**4** in $CDCl_3$

Position	**1**		**2**		**3**		**4**	
	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1	7.16, d (10.2)	157.7	7.16, d (10.2)	158.4	7.11, d (10.2)	157.2	7.09, d (10.2)	156.7
2	5.87, d (10.2)	125.9	5.84, d (10.2)	125.6	5.86, d (10.2)	126.1	5.88, d (10.2)	126.3
3		204.3		204.7		204.1		203.9
4		44.4		44.3		44.2		44.2
5	2.17, dd (12.7, 3.0)	47.0	2.19, m	46.4	2.16, d (13.5)	46.3	2.17, d (13.3)	46.3
6α	1.88, m	24.4	1.96, m	24.0	1.94, m	23.4	1.96, m	23.4
6β	1.88, m		1.79, m		1.81, m		1.80, m	
7	4.71, br s	74.0	5.26 (t-like, 2.6)	74.8	4.53, br s	73.5	4.54, br s	73.4
8		43.5		43.1		42.8		42.9
9	2.59, dd (12.5, 4.1)	40.1	2.20, m	38.7	2.45, dd (12.8, 6.3)	39.6	2.54, dd (12.8, 5.8)	39.6
10		39.9		40.2		40.3		40.3
11α	1.96, m	16.3	1.92, m	16.7	1.98, m	15.1	2.01, m	15.1
11β	1.85, m		1.74, m		1.89, m		1.90, m	
12α	1.80, m	29.4	1.81, m	33.1	1.50, m	25.6	1.41, m	26.0
12β	2.41, m		2.02, m		2.10, m		2.02, m	
13		42.9		47.4		39.7		39.9
14		72.4		159.0		69.9		69.7
15	3.39, s	57.4	5.35, d (1.9)	118.9	3.49, s	57.0	3.54, s	56.9
16α		208.2	2.50, m	34.2		167.7		166.5
16β			2.25, m					
17	3.93, s	50.3	2.89, m	51.0	5.63, s	76.7	5.67, s	75.5
18	1.06, s	25.1	0.85, s	21.2	1.23, s	17.4	1.26, s	18.5
19	1.22, s	20.0	1.19, s	19.2	1.22, s	19.9	1.23, s	19.9
20		132.1		139.6		135.6		145.2
21		174.3		174.8		172.5		169.3
22	7.20, br s	144.5	6.86, s	140.0	7.22, br s	144.6	6.72, s	133.3
23α	4.07, d (20.1)	47.3	3.99, s	46.5	4.06, s	47.1		168.4
23β	4.02, d (20.1)		3.99, s		4.06, s			
28	1.07, s	27.2	1.08, s	27.2	1.06, s	27.3	1.06, s	27.3
29	1.07, s	21.4	1.08, s	21.3	1.07, s	21.4	1.07, s	21.3
30	1.22, s	19.7	1.22, s	27.6	1.16, s	18.5	1.17, s	17.7
7-OAc	2.02, s	169.8	1.96, s	170.2	2.10, s	169.9	2.10, s	169.9
		21.2		21.5		21.2		21.2
N–H	6.31, br s		6.18, br s		6.35, br s		7.50, br s	

17 (δ_C 3.93, s) to C-16 (δ_C 208.2) suggested a carbonyl group at C-16. The cross-peak between δ_C 169.8 and δ_H 4.71 (H-7) demonstrated that the acetoxy group was attached to C-7. Except for the aforementioned data of rings A–D, the molecular formula C_4H_4NO was left. It suggested the presence of a lactam located at C-17, which was confirmed by the HMBC correlations from H-17 (δ_H 3.93) to C-20 (δ_C 132.1), C-21 (δ_C 174.3), C-22 (δ_C 144.5). The NMR resonances associated with the C-17 substituent [C-20 (δ_C 132.1), C-21 (δ_C 174.3), C-22 (δ_C 144.5), C-23 (δ_C 47.3); H-22 (δ_H 7.20, br s), H-23α (δ_H 4.07, d, *J*=20.0 Hz), H-23β (δ_H 4.02, d, *J*=20.0 Hz)] were similar to that of the 3-substituted 1,5-dihydro-2*H*-pyrrol-2-one unit in amooramide A.[14] Moreover, the ^{1}H NMR spectrum of **1** exhibited a resonance characteristic of an amino proton at δ_H 6.31 (br s), which was correlated with C-20, C-21, C-22, and C-23 in the HMBC spectrum. Thus, the planar structure of **1** was determined as depicted in Fig. 1.

The relative configuration of **1** was determined by a ROESY experiment (Fig. 2). The ROESY correlations between H-9/H_3-18, H-5/H-9, H-5/H_3-28 and H-15/H_3-18 showed that H-5, H-9, H-15, Me-18, and Me-28 were on the same face and arbitrarily assigned as α-oriented, whereas the 14,15-epoxide group was β-oriented. The ROESY interactions between H_3-19/H_3-29, Hβ-12/H_3-19, Hβ-12/H-17, Hβ-12/H_3-30, and H-7/H_3-30 suggested the β-orientation of H-7, H-17, Me-19, Me-29, and Me-30. Thus, the structure of **1** was unambiguously determined as depicted.

Toonasinemine B (**2**), an amorphous powder, gave the molecular formula $C_{28}H_{37}NO_4$ on the basis of its $[M+Na]^+$ HRESIMS ion peak at *m*/*z* 474.2619 (calcd for $C_{28}H_{37}NNaO_4$, 474.2615). The ^{1}H NMR and ^{1}C NMR data (Table 1) of **2** were similar to those of **1**. The differences noted between these two limonoids were that **2** had no signals due to 14,15-epoxide and carbonyl group at C-16, but a 14-double bond was indicated by resonances at 159.0 and 118.9 for C-14 and C-15, respectively, and a resonance ascribed to H-15 at 5.64.

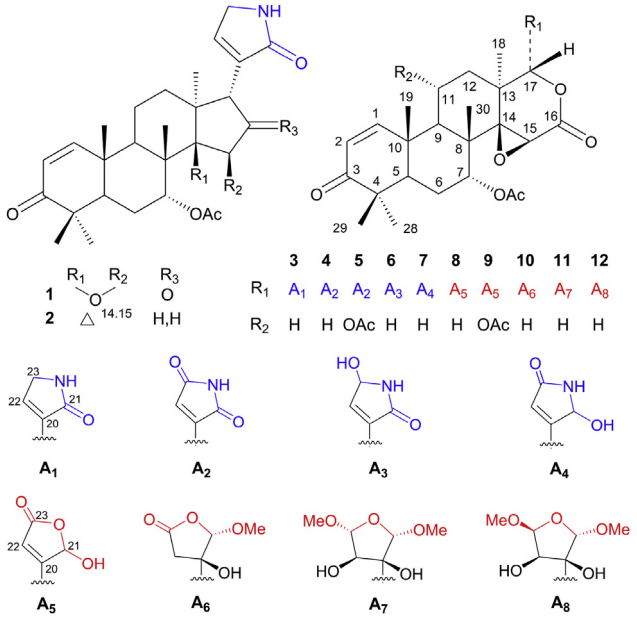

Fig. 1. Chemical structures of compounds **1**–**12**.