Four New Limonoids from the Barks of *Toona ciliata*

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Abstract

Four new limonoids, toonayunnanaes F–I (1–4), and six known compounds (5–10) were isolated from the barks of *Toona ciliata*. Their structures were elucidated by thoroughly analyzing NMR and HRMS data, and single-crystal X-ray diffraction of 1. The oxetane ring moiety in 1 was rare in limonoids and other natural products. Compound 1 showed nitric oxide (NO) inhibitory effect with an IC$_{50}$ 38.45 ± 0.41 µM in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages.

Graphic Abstract

Keywords Meliaceae · *Toona ciliata* · Limonoids · NO inhibitory effects

1 Introduction

The genus *Toona*, belonging to the family Meliaceae, contains 15 species mainly distributed in the regions of tropical Asia and Africa, of which four species and six varieties grow in China [1]. *Toona ciliata* Roem. var. *ciliata* is a timber tree mainly found in the south of China, such as Yunnan, Sichuan, Guangdong, and Hainan provinces [2]. Its bark has been used in Chinese folk medicine to treat dysentery, fever, and menstrual disorders [3]. Our previous phytochemical investigations on *T. ciliata* var. *henryi* and *T. ciliata* var. *yunnanensis* have led to the isolation of a series of limonoids with potential biological activities such as cytotoxicity, anti-inflammatory, and anti-multidrug resistance (MDR) activities [4–8]. As a part of our continuous research for bioactive limonoids from *Toona* plants, four new limonoids, toonayunnanaes F–I (1–4), and six known compounds (5–10) were isolated from the barks of *T. ciliata*. Their structures were elucidated by thoroughly analyzing of NMR and
HRMS data, and single-crystal X-ray diffraction of 1. The oxetane ring moiety in 1 was rare in limonoids and other natural products. Herein, we describe the isolation and structural elucidation of these limonoids as well as their inhibitory effects on NO production in LPS-induced RAW264.7 cells.

2 Results and Discussion

Toonayunnanae F (1), colorless crystals, possessed a molecular formula of C_{26}H_{32}O_{4} according to the HRESIMS ion at m/z 409.2376 [M + H]^+ (calcd. 409.2373). The 1H and 13C NMR data of 1 (Table 1) showed characteristic resonances for a β-substituted furan ring (δH 7.35, 7.16, 6.20; δC 111.2, 124.7, 139.4, 142.8), an α,β-unsaturated ketone (δH 7.08 d,

| No. | δH (ppm) | δC (ppm) | δH (ppm) | δC (ppm) | δH (ppm) | δC (ppm) |
|-----|----------|----------|----------|----------|----------|----------|
| 1   | 7.08 d   | 155.0    | 1.90 m   | 39.1     | 8.00 d   | 160.6    |
| 2   | 5.98 d   | 125.7    | 2.76 m   | 32.9     | 5.84 d   | 124.0    |
| 3   | 204.3    | 44.2     | 217.8    | 204.3    |
| 4   | 2.32 dd  | 44.7     | 2.64 d   | 46.7     | 2.28 dd  | 46.0     |
| 5   | 1.90 m   | 26.3     | 5.31 dd  | 72.2     | 1.98 m   | 23.9     |
| 6   | 4.82 d   | 81.5     | 3.90 br  | 72.1     | 5.27 br  | 73.9     |
| 7   | 148.5    | 41.4     | 1.41 d   | 48.6     | 2.37 d   | 48.8     |
| 8   | 121.4    | 5.66 dd  | 1.56 td  | 17.9     | 4.30 dd  | 67.8     |
| 9   | 2.08 m   | 36.1     | 1.94 m   | 34.5     | 4.03 d   | 77.1     |
| 10  | 51.6     | 96.8     | 2.84 s   | 42.1     | 51.4     | 47.2     |
| 11  | 4.62 br s| 77.7     | 2.50 d   | 221.2    | 5.49 br s| 121.1    |
| 12  | 1.98 m   | 37.9     | 2.50 d   | 43.4     | 2.49 dd  | 36.3     |
| 13  | 3.27 dd  | 43.1     | 3.46 t   | 37.8     | 3.09 dd  | 50.3     |
| 14  | 0.54 s   | 19.1     | 0.78 s   | 27.7     | 0.99 s   | 14.8     |
| 15  | 1.29 s   | 20.9     | 0.84 s   | 18.2     | 1.31 s   | 20.5     |
| 16  | 124.7    | 122.9    | 122.9    | 125.3    |
| 17  | 7.16 s   | 139.4    | 7.26 s   | 140.3    | 7.39 s   | 140.4    |
| 18  | 6.20 s   | 111.2    | 6.27 s   | 110.9    | 6.46 s   | 111.7    |
| 19  | 7.35 s   | 142.8    | 7.39 s   | 143.0    | 7.40 s   | 143.2    |
| 20  | 1.11 s   | 24.8     | 1.24 s   | 31.3     | 1.09 s   | 26.9     |
| 21  | 1.05 s   | 21.6     | 1.05 s   | 19.6     | 1.08 s   | 21.5     |
| 22  | 1.54 s   | 22.3     | 1.14 s   | 17.2     | 1.24 s   | 30.6     |
| 23  | 6-Oac    | 2.13 s   | 21.8     |
| 24  | 6-Oac    | 169.8    |
| 25  | 7-OCH$_3$|          | 3.72 s   |
| 26  | 7-OAc    |          | 1.98 s   |
| 27  | 7-OAc    |          | 21.3     |

Table 1 1H and 13C NMR data of compounds 1–4 in CDCl$_3$ (δ in ppm, J in Hz)

$^a$500 MHz for 1H NMR, 125 MHz for 13C NMR
$^b$600 MHz for 1H NMR, 150 MHz for 13C NMR
$J = 10.4 \text{ Hz}, \delta_C 125.7, 155.0, 204.3$), and five singlet methyl groups ($\delta_H 0.54, 1.05, 1.11, 1.29, 1.54$). Those observations together with 26 carbon resonances and 11 indices of hydrogen deficiency, suggested that 1 might be a ring-intact limonoid with an $\alpha,\beta$-unsaturated carbonyl moiety in A ring [8–10] (Fig. 1).

The double bond between C-9 and C-11 was confirmed by the correlations of $H_3-19$ ($\delta_H 1.29$) and $H_3-30$ to C-9 ($\delta_C 148.5$); of $H-11$ ($\delta_H 5.66$) to C-12 ($\delta_C 36.1$); and of $H_2-12$ ($\delta_H 2.08$) to C-9 and C-11 ($\delta_C 121.4$) in the HMBC spectrum. The HMBC correlations (Fig. 2) from $H-7$ ($\delta_H 4.82$) to C-8 ($\delta_C 47.1$) and C-6 ($\delta_C 26.3$); from $H_3-30$ ($\delta_H 1.54$) to C-7 ($\delta_C 81.5$) and C-14 ($\delta_C 96.8$); from $H_3-18$ ($\delta_H 0.54$) and H-15 ($\delta_H 4.62$) to C-14; and from H-16 and H-17 to C-15 ($\delta_C 77.7$) indicated that C-7, C-14, and C-15 were all oxygenated. The significantly deshielded chemical shift of C-14 and the indices of hydrogen deficiency suggested the presence of an oxetane ring between C-7 and C-14 [8, 11, 12] and a hydroxy group at C-15, similar to that in ciliatasecone S [8]. Therefore, the 2D structure of 1 with a rare oxetane ring was constructed. The ROESY cross-peaks of $H_3-19/H_3-29, H_3-19/H_3-30$, and $H_3-30/H-7$ (Fig. 2) revealed the $\beta$-orientations of Me-19, Me-29, Me-30, and H-7. The $\alpha$-orientations of H-5, Me-18, and Me-28 were determined by the ROESY cross-peaks of H-5/H_3-28 and H-5/H_3-18. The cross-peaks of $H_3-18/H-16$ ($\delta_H 1.98$), H-16 ($\delta_H 1.98$)/H-15, and H-16

Fig. 1 The structures of compounds 1 – 10

Fig. 2 The key HMBC and ROESY correlations and X-ray crystallographic structure of compound 1
(δH 1.87)/H-17 indicated the β-orientation of 15-OH and the α-orientation of furan ring. A single-crystal X-ray crystallographic diffraction experiment with Cu Kα radiation of 1 (Fig. 2) unambiguously determined the α-orientation of the oxetane ring moiety and the absolute configuration as 5R, 7R, 8S, 10R, 13S, 14S, 15R, and 17S. The structure of compound 1 was thus established as shown.

The molecular formula of toonayunnanae G (2) was established as C28H38O6 based on the HRESIMS ion at m/z 493.2551 [M + Na]+ (calcd. 493.2561) and its 13C NMR data. Apart from the signals for an acetoxy substituent, the remaining 26 carbon resonances observed in the 1D NMR spectra indicated that 2 might also be a ring-intact limonoid [8–10]. Comparison of its NMR data (Table 1) with those of the known toonaciliatone F (5) [13] revealed that the main differences were the absence of a double bond and an acetoxy group. The chemical shift of C-3 (δC 217.8) implied the presence of a keto carbonyl rather than an α,β-unsaturated ketone moiety in ring A, which was confirmed by the HMBC correlations of H2-1 (δH 1.90, 1.77), H2-2 (δH 2.76, 2.31), H3-28, and H3-29 to C-3. The HMBC correlations of H-7 (δH 5.31) to C-6, C-8, C-9, and C-30, and an ester carbonyl carbon (δC 169.8) indicated that an acetoxy group was located at C-7. The hydroxy groups at C-11 and C-12 were revealed by the HMBC correlations (Fig. 3) of H-11 (δH 4.30) to C-9 and C-12 (δC 77.1) and of H-12 (δH 4.03) to C-11 (δC 67.8) and C-13. The ROESY cross-peaks of H3-30/H-7, H3-30/H-11, H-11/H-12, and H-12/H-17 (Fig. 3) suggested the α-orientations of 7-OAc, 11-OH, and 12-OH. Therefore, the structure of 3 was determined as shown.

The clearly diagnostic signals (Table 1) for a β-substituted furan moiety, five characteristic singlet methyl groups in the 1D NMR spectra, and the correlations of a methoxy group (δH 3.72) with an ester carbonyl carbon (δC 170.8) in the HMBC spectrum suggested that toonayunnanae I (4) was likely a B-seco limonoid with a C6-C7-methyl ester appendage [5, 8, 14–16], similar to toonayunnanin G (6) [15]. Comparison of the 1D NMR
data (Table 1) of 4 with those of 6 revealed that the major difference was the presence of a doublet methyl signal (δH 1.06, 3H, d, J = 5.8 Hz), which was ascribed to Me-28 at C-4 based on its HMBC correlations (Fig. 3) with C-3 (δC 210.1), C-4 (δC 41.8), and C-5 (δC 51.2). Therefore, 4 possessed a B-seco-29-nor-limonoid skeleton similar to that of ciliatasecone N [8]. The HMBC correlations of H-12 (δH 4.23) to C-11 (δC 79.3) and C-13 indicated that a hydroxy group was located at C-12. The relative configuration of 4 was deduced to be identical to that of 6 by a ROESY experiment. The a-orientations of 12-OH and Me-28 in 4 was inferred from the ROESY cross-peaks (Fig. 3) of H-11/H-12, H-12/H-17, and H-28/H-5. Accordingly, the structure of 4 was elucidated as shown.

By comparing their 1H and 13C NMR spectroscopic data with those reported in literatures, six known compounds were identified as toonaciliatone F (10), toonaciliatin P (11), toonacilianin C (12), toonacilianin H (13), toonacilianin G (14), and toonacilianin D (15), toonacilianin G (16), toonacilianin F (17), toonacilianin E (18).

Additionally, the inhibitory effects on NO generation in LPS-activated RAW 264.7 macrophages of compounds 1 – 4 were evaluated at the concentrations of 50 μM and below. Compound 1 showed NO inhibitory effect with an IC50 38.45 ± 0.41 μM.

3 Experimental

3.1 General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 automatic digital polarimeter at room temperature. IR spectra were recorded on a Bruker Tensor 27 spectrometer using KBr pellets. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). High-resolution electrospray ionization mass spectrometry (HRESIMS) was obtained on an Agilent 6529B Q-TOF mass instrument using electrospray ionization. The 1D and 2D nuclear magnetic resonance (NMR) spectra were obtained on Bruker AVANCE III 500 MHz or Bruker AVIII HD 600 MHz spectrometers in CDCl3 with TMS as an internal standard. Analytical HPLC was conducted on an Agilent 1260 infinity system equipped with a DAD-UV detector. Preparative HPLC was carried out using a Shimadzu LC-6A 1260 infinity system equipped with a DAD-UV detector. IR spectra were recorded on a Bruker Tensor 27 spectrometer using KBr pellets. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). Optical rotations were measured on a JASCO P-1020 automatic digital polarimeter at room temperature. IR spectra were recorded on a Bruker Tensor 27 spectrometer using KBr pellets. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan).

3.2 Plant Material

The air-dried bark of Toona ciliata Roem. var. ciliata was collected in Baoshan, Yunnan Province, China, in August 2018. The plant material was identified by professor Mian Zhang of the research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (no. 2018-TC) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3 Extraction and Isolation

The air-dried and powder bark of T. ciliata (29 kg) was extracted with 95% EtOH three times (3 × 6.5 L) under reflux. The concentrated extract (3.1 kg) was suspended in H2O, and then partitioned with petroleum ether (PE) and dichloromethane (DCM), successively. The DCM extract (100.0 g) was subjected to a silica gel column (200 – 300 mesh) eluted with a PE-DCM mixture (100:0 – 100:1) in a step gradient to obtain eight major fractions (A – H). Fraction C (20.0 g) was loaded onto an ODS column eluted with a mixture of MeOH-H2O from 40 to 70% to afford four fractions (C1 – C4). Fraction C4 (3.3 g) was further applied to an ODS column (30% – 60% ACN-H2O) to give four subfractions (C4a – C4d). Fraction C4a (374 mg) was separated by preparative HPLC with 60% MeOH-H2O to yield 1 (4 mg) and 2 (11 mg). Similarly, fraction C4c (582 mg) afforded 3 (5.3 mg), 4 (14 mg), and 8 (10.3 mg) by preparative HPLC with 50% ACN-H2O. Fraction D (15 g) was subjected to an MCI column (50% – 70% MeOH-H2O) to give three subfractions (D1 – D3). Fraction D2 (5.5 g) was separated by an ODS MPLC (40% – 60% ACN-H2O) to afford five subfractions (D2a – D2e), and fraction D2b (539 mg) was purified by preparative HPLC with MeOH-H2O to give 6 (5.2 mg) and 9 (14 mg). Using the same purification procedures, fraction D3 (3.3 g) was further fractionated by ODS column chromatography (40% – 60% MeOH-H2O), and the subfraction D3c (483 mg) was purified by preparative HPLC with 45% ACN-H2O to give 4 (2.2 mg), 7 (34.1 mg), and 10 (45 mg).

Toonayunnanae F (1): colorless crystals (MeOH-H2O); [α]D24 + 7.6 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 218 (5.30) nm; IR (KBr) νmax 3553, 2986, 2931, 2859, 1669, 1455, 1387, 1158, 1025 cm−1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 409.2376 [M + H]+ (calcd. for C26H33O4, 409.2373).

Toonayunnanae G (2): white amorphous powder; [α]D24 + 9.2 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 208 (3.95) nm; IR (KBr) νmax 3409, 2964, 1724, 1384, 1247, 1028 cm−1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 493.2551 [M + Na]+ (calcd. for C26H38NaO6, 493.2561).
Toonayunnanae H (3): white amorphous powder; [α]D 24 + 7.6 (c 0.1, MeOH); UV (MeOH) λ max (log ε) 208 (3.55) nm; IR (KBr) ν max 3421, 2977, 1732, 1664, 1249, 1027 cm−1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 469.2577 [M + H]+ (calcd. for C28H37O6, 469.2585).

Toonayunnanae I (4): white amorphous powder; [α]D 24 − 30.2 (c 0.1, MeOH); UV (MeOH) λ max (log ε) 208 (4.02) nm; IR (KBr) ν max 3466, 2954, 1747, 1717, 1226, 1026 cm−1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 550.2631 [M + NH4]+ (calcd. for C28H40NO10, 550.2647).

3.4 Anti-inflammatory Activities

The new compounds (1–4) were evaluated for their inhibitory effects on NO production in LPS-activated RAW 264.7 macrophages as described in the literature [19]. Briefly, RAW 264.7 cells (6 × 106 cells/mL) were seeded in 96-well plates and treated with different concentrations of tested compounds for 1 h, and 1.0 μg/mL LPS solution was subsequently added to stimulate the cells for 18 h. NO level was evaluated by measuring the standard of accumulated nitrite in cell supernatants with the reagent of Griess. N-Monomethyl-L-arginine Monoacetate (L-NMMA) was used as a positive control (IC50 = 42.38 ± 0.72 μM).

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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