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# Ciliatonoids A and B, Two Limonoids from *Toona ciliata*

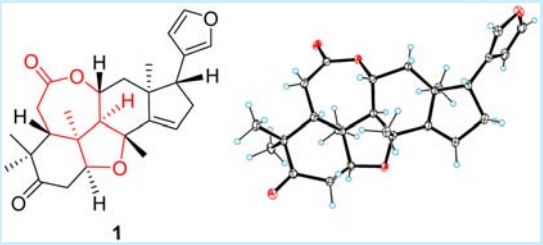
Cui-Ping Liu,[†] Guo-Cai Wang,[†] Li-She Gan,[‡] Cheng-Hui Xu,[†] Qun-Fang Liu,[†] Jian Ding,[†] and Jian-Min Yue*[,†]

[†]State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, People's Republic of China

[‡]College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

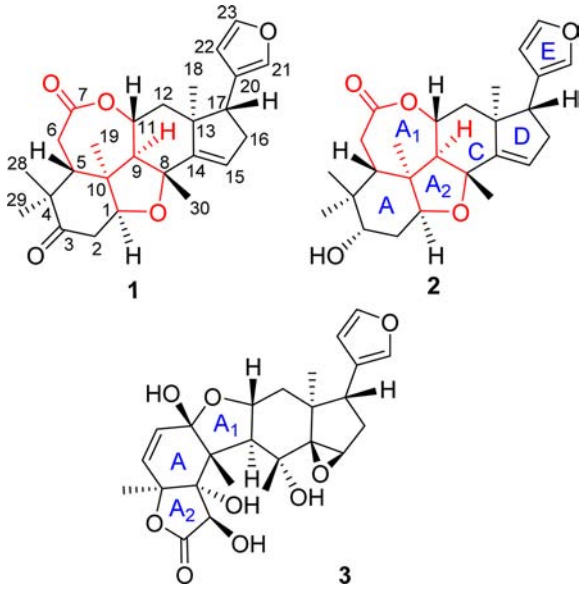
Supporting Information

**ABSTRACT:** Three new ring B-*seco* limonoids, ciliatonoids A–C (**1**–**3**), were isolated from *Toona ciliate* and structurally characterized by spectroscopic data, X-ray crystallography, and electronic circular dichroism analysis. Ciliatonoids A and B feature an unprecedented limonoid architecture, while ciliatonoid C belongs to a rare class of limonoids. Biological evaluation showed that compound **3** exhibited modest activities against the tested tumor cell lines.




The genus *Toona* (Meliaceae family) comprising 15 species mainly distribute in the regions of Asia and Africa, of which four species and six variants grow in China.[1] These woody plants are well-known for their applications in food and medicine and have attracted interest from the natural products related communities, which led to the isolation of a wide array of compounds, in particular, the limonoids with diverse biological activities such as cytotoxic, antifungal, antifeedant, and antiulcer effects.[2] *Toona ciliata* Roem, a timber tree, is mainly found in the southern Provinces of China.[3] The plant parts have long been used for the treatment of dysentery, fever, and menstrual disorders in Chinese folk medicine.[4] As a continuation of chemical studies on the plants of *Toona* genus,[5] three new ring B-*seco* limonoids, ciliatonoids A–C (**1**–**3**), were isolated from an ethanolic extract of the twigs of *T. ciliate*. Their structures with the absolute stereochemistry were characterized by spectroscopic data, X-ray crystallography, and electronic circular dichroism (ECD) analysis. Ciliatonoids A and B featured an unprecedented limonoid architecture by furnishing a very unique *cis*-fused central motif (in red) of methylhexahydro-3*H*,6*H*-furo[3,4-*c*]oxepin-6-one, while compound **3** belonged to a rare class of limonoids and showed modest activities against the tested tumor cell lines. We herein present the isolation, structural elucidation, and cytotoxic evaluation of compounds **1**–**3**.

Ciliatonoid A (**1**), colorless crystals, possessed a molecular formula $C_{26}H_{32}O_5$ as determined by HRESIMS at *m*/*z* 447.2141 $[M + Na]^+$ (calcd 447.2147), requiring 11 degrees of unsaturation. The $^{13}C$ NMR spectrum (Table 1) resolved 26 carbon resonances corresponding to 5 methyl, 4 methylene, 9 methine (four olefinic and two oxygenated), and 8 quaternary (one oxygenated, two olefinic, and two carbonyl) carbons as distinguished by the HSQC spectrum and DEPT experiments (Figures S3 and S4). In addition, five tertiary methyls ($\delta_H$ 1.10, 1.13, 1.20, 1.24, and 1.51), one trisubstituted double bond ($\delta_H$ 5.80; $\delta_C$ 122.2 and 156.5), and a $\beta$-substituted furan ring ($\delta_H$ 6.24, 7.27, and 7.40) were identified by NMR data (Table 1) analysis. The aforementioned data and biogenetic consideration suggested that compound **1** possessed the limonoid characteristics.




Comprehensive investigation on the 2D NMR spectra of **1**, especially HMBC data (Figure 1A and Figure S5), allowed the establishment of its planar structure. Briefly, the HMBC correlations from H-17 to C-20, C-21, and C-22 placed the furan ring at C-17. A $\Delta^{14}$ double bond was located on the basis of the chemical shifts of C-14 and C-15 and multiple HMBC

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**Table 1. NMR Data of Compounds 1−3**

| | 1[a] | | 2[a] | | 3[b] | |
|---|---|---|---|---|---|---|
| no. | $\delta_H$ (multi, *J*, Hz) | $\delta_C$ | $\delta_H$ (multi, *J*, Hz) | $\delta_C$ | $\delta_H$ (multi, *J*, Hz) | $\delta_C$ |
| 1 | 4.24, dd (10.2, 7.6) | 88.5 | 4.29, dd (10.2, 7.6) | 87.5 | | 105.7 |
| 2a | 2.98, m | 44.3 | 2.10, m | 33.6 | 6.22, d (10.5) | 134.3 |
| 2b | 2.92, m | | 1.99, m | | | |
| 3 | | 212.9 | 3.65, dd (9.7, 3.5) | 74.4 | 5.98, d (10.5) | 126.4 |
| 4 | | 47.2 | | 38.3 | | 87.4 |
| 5 | 3.04, m | 42.8 | 2.37, t (9.0) | 41.6 | | 82.5 |
| 6a | 3.10, m | 34.5 | 2.91, m | 35.9 | 4.72, s | 78.3 |
| 6b | 2.95, m | | | | | |
| 7 | | 172.9 | | 173.9 | | 174.7 |
| 8 | | 83.6 | | 82.6 | | 76.0 |
| 9 | 2.95, m | 56.5 | 2.94, m | 56.3 | 3.47, d (12.0) | 56.0 |
| 10 | | 44.0 | | 41.7 | | 55.3 |
| 11 | 4.90, ddd (12.0, 9.4, 2.0) | 73.2 | 4.87, ddd (12.5, 10.0, 2.3) | 73.3 | 4.90, ddd (12.0, 9.0, 7.0) | 74.5 |
| 12$\alpha$ | 2.12, dd (15.3, 2.0) | 40.4 | 2.05, m | 40.5 | 1.75, dd (13.0, 9.0) | 44.5 |
| 12$\beta$ | 2.26, dd (15.3, 9.4) | | 2.25, dd (15.5, 10.0) | | 2.68, dd (13.0, 7.0), | |
| 13 | | 50.8 | | 50.8 | | 41.9 |
| 14 | | 156.5 | | 158.1 | | 73.6 |
| 15 | 5.80, d (3.8) | 122.2 | 5.75, d (3.5) | 121.5 | 3.59, s | 56.5 |
| 16$\alpha$ | 2.61, ddd (15.7, 10.8, 1.5) | 35.4 | 2.59, dd (15.7, 11.0) | 35.4 | 1.86, dd (13.5, 11.4) | 31.8 |
| 16$\beta$ | 2.47, ddd (15.7, 6.8, 3.8) | | 2.44, ddd (15.7, 6.6, 3.5) | | 2.16, dd (13.5, 6.5) | |
| 17 | 2.89, m | 52.8 | 2.87, m | 52.7 | 2.93, dd (11.4, 6.5) | 42.3 |
| 18 | 1.10, s | 24.9 | 1.10, s | 25.6 | 1.25, s | 23.7 |
| 19 | 1.24, s | 27.5 | 1.24, s | 26.1 | 1.99, s | 13.7 |
| 20 | | 123.8 | | 123.9 | | 123.8 |
| 21 | 7.27, s | 140.1 | 7.25, s | 140.1 | 7.43, s | 140.5 |
| 22 | 6.24, s | 110.6 | 6.23, s | 110.7 | 6.38, s | 111.7 |
| 23 | 7.40, s | 143.3 | 7.39, s | 143.2 | 7.67, s | 143.9 |
| 28 | 1.13, s | 28.4 | 1.01, s | 27.1 | 2.00, s | 24.7 |
| 29 | 1.20, s | 19.7 | 0.91, s | 15.2 | | |
| 30 | 1.51, s | 29.4 | 1.42, s | 29.7 | 1.69, s | 24.4 |
| HO-5 | | | | | 6.38, s | |
| HO-8 | | | | | 8.19, s | |

[a]Recorded in $CDCl_3$ at 400 MHz for $^1H$ NMR and 125 MHz for $^{13}C$ NMR. [b]Recorded in $C_5D_5N$ at 400 MHz for $^1H$ NMR and 125 MHz for $^{13}C$ NMR.

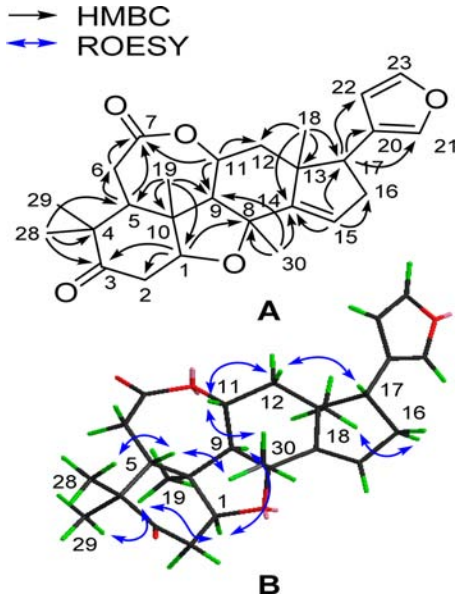


**Figure 1.** Key HMBC (A) and ROESY (B) correlations of **1**.

correlations of H-15/C-14, C-16, and C-17 and $H_3$-18 and $H_3$-30/C-14. The chemical shifts of C-1 ($\delta_C$ 88.5) and C-8 ($\delta_C$ 83.6), as well as the key HMBC correlation from H-1 to C-8, indicated a linkage between C-1 and C-8 via an oxygen atom. Additionally, the key HMBC correlations from H-5, H-6, and H-11 to C-7 revealed the presence of a seven-membered lactone ring. Finally, a 3-keto group was assigned by the chemical shift and HMBC correlations of H-1 and H-28/C-3 ($\delta_C$ 212.9). The planar structure of **1** was thus delineated as an unprecedented architecture of B-*seco* limonoid.

The relative configuration of **1** was established by the analysis of its ROESY spectrum (Figure 1B and Figure S6). The key ROESY correlations of H-18/H-16$\alpha$ and H-17/H-12$\beta$ suggested that $CH_3$-18 and the furan ring were $\alpha$-oriented. The ROESY cross-peaks of H-11/H-12$\beta$ and H-30, and H-5/H-30 and H-28 indicated that H-5, H-11, $CH_3$-28, and $CH_3$-30 were $\beta$-configured. Consequently, H-1/H-9 and H-19, and H-19/H-29 indicated that they adopted $\alpha$-orientation. Fortunately, qualified colorless crystals of **1** were obtained by recrystallization in MeOH, which allowed a successful performance of X-ray crystallography by employing graphite-monochromated Cu K$\alpha$ radiation ($\lambda$ = 1.54178 Å). The single-crystal X-ray diffraction study (Figure 2 and Table S1) not only confirmed the structure of **1** as assigned by spectroscopic data



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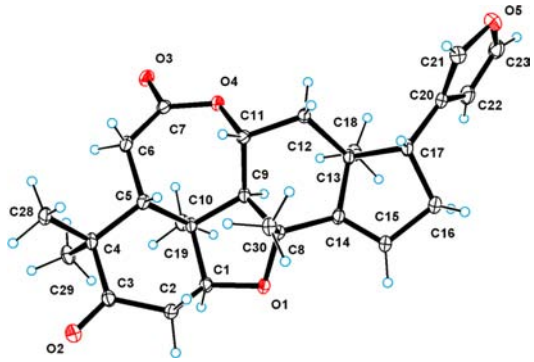


**Figure 2.** ORTEP drawing of compound **1**.

analysis but also determined its absolute configuration [absolute structure parameter: 0.14 (19)][6] as shown, which is consistent with the biogenetic considerations for limonoids.

Ciliatonoid B (**2**) was assigned a molecular formula $C_{26}H_{34}O_5$ based on the sodiated molecular conjugate ion peak at $m/z$ 875.4703 $[2\ M + Na]^+$ (calcd 875.4710) in the HRESIMS, which showed two hydrogen atoms more than **1**. The NMR data (Table 1) of **2** showed high similarities to those of **1**, except for the presence of an oxygenated methine in **2** in place of the C-3 keto carbonyl of **1**. The chemical shift of C-3 ($\delta_C$ 74.4) and the key HMBC correlations from H-1, H-2, and H-28 to C-3 (Figure S13) indicated the presence of a hydroxy group at the C-3 of **2**, which is consistent with its molecular formula. The relative configuration of **2** was established by ROESY experiments (Figure S14) in which the key correlation between H-3 and H-5 indicated that the HO-3 was $\alpha$-oriented. The relative configurations of the other stereocenters in compound **2** were assigned to be the same as those of **1** based on the similar NMR data and ROESY correlations. Furthermore, the absolute stereochemistry of **2** was determined as depicted by comparing the ECD spectrum with that of **1** (Figure 3). The ECD curves of **2** well matched those of **1** in the

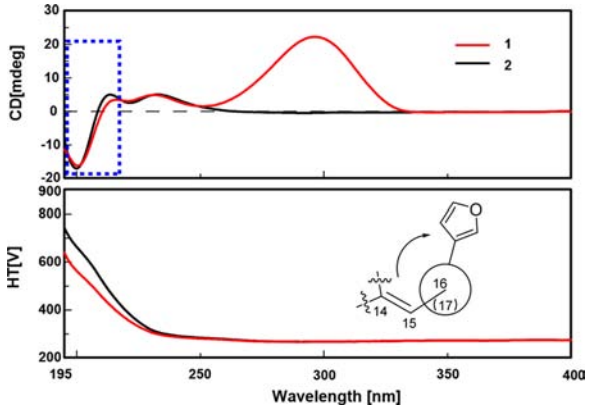


**Figure 3.** ECD spectra of compounds **1** and **2**; partial stereoview of **1** and **2** along the C-16−C-17 bond. Arrow denotes the electric transition dipole of two chromophores.

region 195 to 250 nm, suggesting that they shared the same absolute stereochemistry. The structure of compound **2** was thus assigned as shown.

The CD spectra of compounds **1** and **2** showed the split patterns around 209 nm (Figure 3). The positive Cotton effects at $\lambda$max 212 nm ($\Delta\varepsilon$ +1.2 for **1**, +1.6 for **2**) and the negative Cotton effects at 200 nm ($\Delta\varepsilon$ −5.2 for **1**, −5.5 for **2**) arising from the exciton coupling of two different chromophores of $\beta$-substituted furan ring and the $\Delta^{14}$ double bond indicated a positive chirality for both compounds **1** and **2**, which is consistent with the absolute configuration of **1** as established by X-ray data. The large positive absorption at about 300 nm in the ECD spectrum of **1** is likely resulted from the n→$\pi^*$ transition interaction of the C-3 keto group.

Ciliatonoid C (**3**) had a molecular formula of $C_{25}H_{30}O_9$ as determined by the HRESIMS ion peak at $m/z$ 497.1775 $[M + Na]^+$ (calcd 497.1788). The $^{13}C$ NMR data (Table 1) showed 25 carbon resonances corresponding to 4 methyl, 2 methylene, 10 methine (five olefinic and three oxygenated), and 9 quaternary (one ester carbonyl, one olefinic, one ketal, and four oxygenated) carbons. Comprehensive analysis of the NMR data suggested that compound **3** was a norlimonoid that possessed a structure similar to that of toonaciliatin J[5a] except for the absence of the OH-12. This was confirmed by analysis of its HMBC spectrum (Figure 4) in which the key HMBC

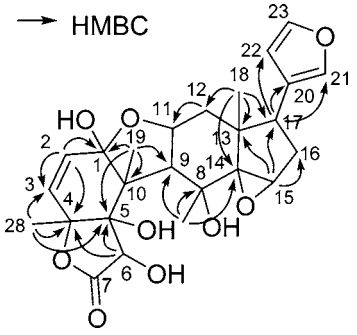


**Figure 4.** Key HMBC correlations of **3**.

correlation network of $H_3$-18/C-12, C-13, C-14, and C-17 was observed, and this is consistent with the chemical shift of C-12 ($\delta_C$ 44.5). The HMBC correlations from H-17 to C-20, C-21, and C-22 placed the furan ring to C-17. Although no HMBC correlation is available, the chemical shifts of C-1 ($\delta_C$ 105.7) and C-11 ($\delta_C$ 74.5) indicated the presence of an oxygen bridge between C-1 and C-11. The locations of the other functional groups and linkages of the remaining subunits were also verified by HMBC correlations (Figure 4). The relative configuration of **3** was assigned the same as toonaciliatin J by the similar NMR data, particularly the very similar coupling patterns in the $^1H$ NMR spectra of both compounds. Compound **3** was thus assigned as 12-deoxytoonaciliatin J and was given the trivial name ciliatonoid C.

The absolute configuration of compound **3** was assigned by quantum chemical time-dependent density functional theory (TDDFT) calculation of its theoretical ECD spectrum. In this calculation, two lowest energy conformers due to the free rotation of the C-17/C-20 bond at the energy window of 2 kcal/mol were taken into consideration (Supporting Information, S3). The calculated ECD spectrum showed good consistency with the experimental ECD curves at the region of 190−220 nm (Figure 5), both of which showed a dominated positive Cotton effect resulting from the interaction between the chromophore furan ring and the adjacent chiral centers. Therefore, the absolute configuration of compound **3** was established as shown, which is consistent with those of all the limonoids identified up to now from biosynthetic aspects. In contrast to compounds **1** and **2**, compound **3** only showed a


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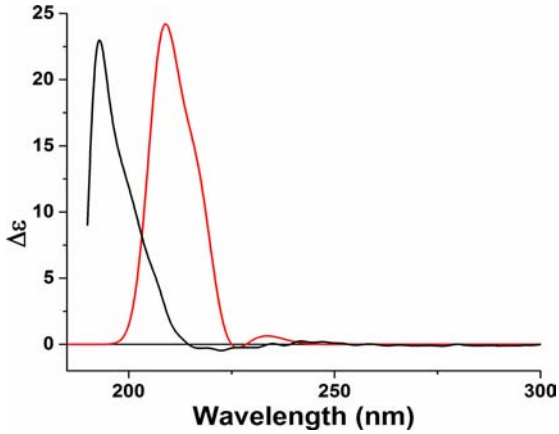


**Figure 5.** Experimental (black line) and B3LYP/6-311++G(2d,2p)//B3LYP/6-31+G(d) calculated (red line) ECD spectra of **3**.

simple positive Cotton effect duo likely to its different scaffold and the absence of the $\Delta^{14}$ double bond.

Compounds **1**−**3** were tested for the cytotoxicity against HL-60 (human premyelocytic leukemia), P-388 (murine leukemia), and A-549 (human lung adenocarcinoma) tumor cells and with adriamycin as the positive control ($IC_{50}$ = 0.131 $\mu$M against HL-60, 0.545 $\mu$M against P-388, and 0.324 $\mu$M against A-549). In these tests, the HL-60 and P-388 cell lines were tested by using the microculture tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method,[7] and the A-549 cell line was evaluated by using the sulforhodamine B (SRB) method.[8] Only compound **3** displayed modest cytotoxicity against HL-60 and P-388 with $IC_{50}$ values of 1.19 and 2.50 $\mu$M, respectively.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01213.

Experimental section and raw spectroscopic data including IR, MS, and NMR spectra for compounds **1**−**3**; ECD calculation for **3** (PDF)

X-ray data for **1** (CIF)

## ■ AUTHOR INFORMATION

### Corresponding Author

*E-mail: jmyue@simm.ac.cn.

### Notes

The authors declare no competing financial interest.

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