Limonoids from the Leaves and Stems of *Toona ciliata*

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Three new norlimonoids, toonaciliatins A (**1**), F (**6**), and G (**7**), four new limonoids, toonaciliatins B−E (**2**−**5**), and five known compounds, 5α,6*β*,8α-trihydroxy-28-norisotoonafolin (**8**), toonaciliatins H (**9**) and I (**10**), febrifugin (**11**), and khayasin T (**12**), were isolated from the leaves and stems of *Toona ciliata*. Compounds **1**−**3** have an unusual 1-en-3-one system with a 1,11-oxygen bridge, limonoids **11** and **12** have a mexicanolide-type structural frame, and others are typical A,B-*seco*- (**4**, **5**, **9**, and **10**) or B-*seco*-29-nor- (**3**, **6**, **7**, and **8**) limonoids. Toonaciliatins H (**9**) and I (**10**) were obtained for the first time as natural products. The structures were established by extensive spectroscopic methods, particularly 1D and 2D NMR techniques.

Limonoids are secondary metabolites characteristic of the plant family Meliaceae, from which many structurally diversified and chemosystematically significant limonoids have been isolated in the past several decades.[1−5] The evolutionary trends of limonoid chemistry corresponding to the morphological division of Meliaceae have been discussed in detail.[2] It was demonstrated that genera of the subfamily Melioideae contain limonoids formed via several different biosynthetic routes and characterized by relatively low skeletal specializations and oxidation states, while genera of the subfamily Swietenioideae generally produce limonoids through a unique major biosynthetic route and have relatively high skeletal specializations and oxidation states. Insecticidal, insect antifeedant and growth regulating activity, antibacterial, antifungal, antimalarial, anticancer, and antiviral activities have been reported for many liminoids.[3,5] These activities, particularly the pronounced insecticidal and antifeedant activities, have prompted many efforts toward use of limonoids or their derivatives for specific agricultural or medicinal applications.[3,5] *Toona* was originally described by Endlicher (1840) as a section of *Cedrela* but was later raised to generic rank by Roemer (1846) and was included in the subfamily Swietenioideae by Pennington and Styles (1975).[6] Studies of *Toona ciliata* Roem. (Meliaceae), also known as *Cedrela toona* Roxb. and *Toona sureni* (Bl.) Merr.,[7] have improved knowledge of the genus *Toona* from the taxonomic aspect. The limonoid chemistry of *T. ciliata* was studied extensively,[6,8−18] and an earlier discussion of chemosystematic and ecological significance suggested *Toona* to be peripheral Melioideae as well as Swietenioideae.[2] Further investigations[6,16−18] suggested that the limonoids isolated from *T. ciliata* did not support the affiliation of *Toona* to the subfamily Swietenioideae but to Melioideae[6,16−18] and that the limonoids were related to the phytochemical resistance of *T. ciliata* to the shoot borer *Hypsipyla*.[16] Our investigation of the limonoids of the leaves and stems of this species provides further understanding of its taxonomic position in the Meliaceae.

Results and Discussion

Separation of the EtOAc-soluble fraction of an EtOH extract through MCI column chromatography eluted with MeOH/H_2O (4/6, 5/5 to 8/2, and 9/1) afforded, respectively, three major fractions 1, 2, and 3. Extensive column chromatography of fractions 2 and 3 furnished 12 limonoids and norlimonoids (**1**−**12**).Toonaciliatin A (**1**) showed a molecular formula of $C_{25}H_{28}O_{10}$ as determined by the HREIMS ion peak at *m*/*z* 488.1700 (calcd 488.1682). The positive-mode (*m*/*z* 489.0 $[M + H]^+$; 999.2 $[2M + Na]^+$) and negative-mode (*m*/*z* 487.2 $[M - H]^-$, 975.7 $[2M - H]^-$) ESIMS further supported this assignment. IR absorption bands at 3483, 1782, and 1628 cm^{-1} suggested the presence of hydroxyl, *γ*-lactone,[18] and carbon−carbon double-bond functionalities. Observations of proton signals for a *β*-substituted furan ring and four methyl singlets (Table 1) in the ^1H NMR spectrum and 25 carbon signals in the ^{13}C NMR spectrum suggested that **1** was a norlimonoid. Comparison of the ^1H and ^{13}C NMR data (Tables 1 and 3) with those of 5α,6*β*,8α-trihydroxy-28-norisotoonafolin (**8**), also isolated from the seeds of this species[18] and in this investigation, indicated that **1** and **8** had similar B′CDE ring systems with only the signals around CH-11 being downfield shifted. The signals for ring A [δ_C 191.8 (C), 97.7 (CH), 193.8 (C), and 86.3 (C); δ_H 5.40, s, 1H] suggested that, instead of a 1-oxygen-3-one system, **1** possessed a unique 1-oxygen-1-en-3-one system in ring A possibly resulting from a 1,3-diketone. The presence of such a functional group was also supported by a UV absorption maximum at 268 nm.[19] The stability of this system was assumed to be the formation of an ether linkage at C-1 or C-3. The markedly downfield shifted signals at CH-11 (δ_C from 79.7 to 85.3 and δ_H from 4.22 to 4.82) supported this assumption and established the 1,11-oxygen bridge. HMBC correlation analysis (Figure 1) supported the proposed structure for **1**. In particular, correlations of H-2 with C-1, C-4, and C-10, of H_3-28 with C-3, C-4, and C-5, and of H_3-19 with C-1, C-5, C-9, and C-10 constructed the ring A system as depicted. The relative stereochemistry of **1** was determined by analysis of its NOESY spectrum (Figure 1) and proton coupling constants. The absence of a NOESY correlation between H_3-19 and H-6 and observation of NOESY correlations between H_3-28 and H-6 indicated that both H-6 and H_3-28 were α-oriented. The *β*-configuration of H-11 was indicated by the NOESY correlation between H-11 and H_3-19, while the NOESY correlation between H-12 and H-17 and the small coupling constant ($J = 4.8$ Hz) between H-12 and H-11 required H-12 to be on the *β* and OH-12 on the α side of the molecule.[18] The configuration of the 14,15-epoxide was indicated by the NMR data of **1** and **8**, which showed similar chemical shifts and proton coupling patterns around this group. This conclusion was verified by a Chem 3D-generated molecular model, in which the spatial proximity of H-16α and H_3-18 was consistent with the NOESY correlation observed between the protons (an α-oriented 14,15-

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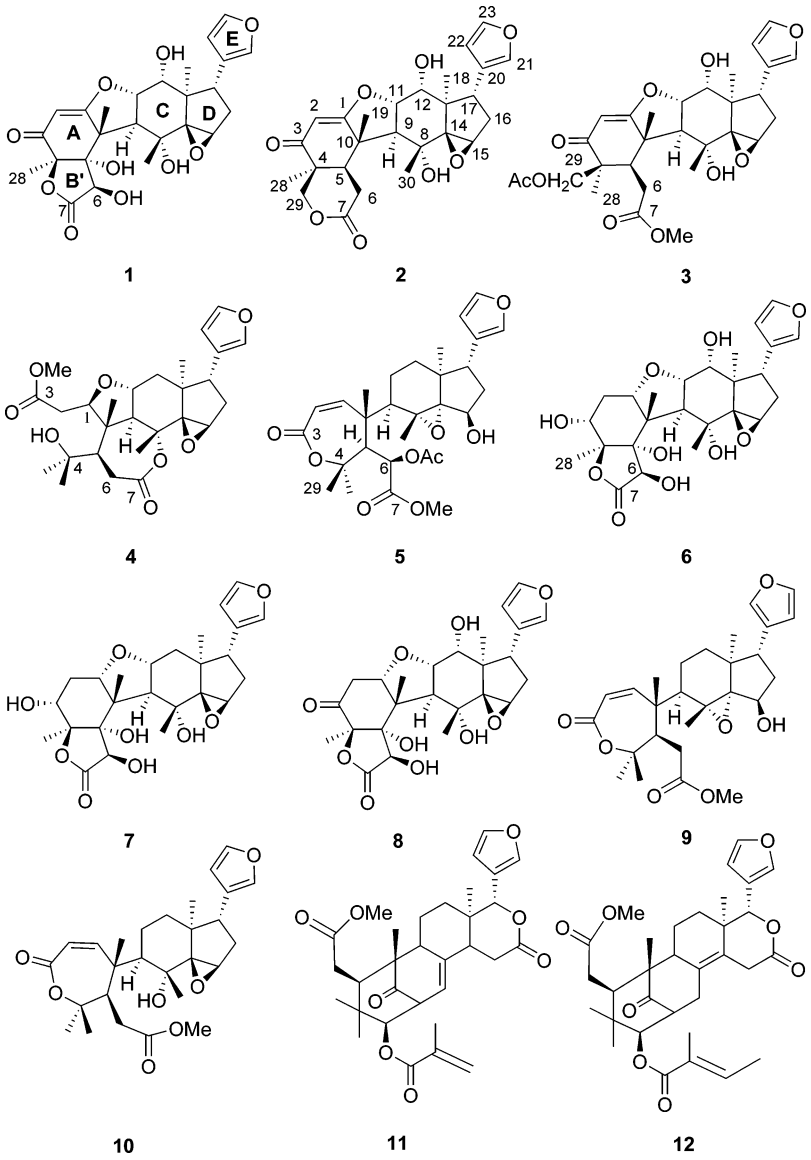
Chart 1



epoxide would draw away these protons). The structure of **1** was thus established as depicted.

The molecular formula $C_{26}H_{30}O_8$ was determined for toonaciliatin B (**2**) by the HREIMS peak at *m*/*z* 470.1962 (calcd 470.1941). The UV spectrum of **2** was very similar to that of **1**. However, the absence of an IR absorption band at ca. 1780 cm^{-1} indicated that the five-membered 4,7-lactone did not exist in **2**. Comparison of the ^1H and ^{13}C NMR data (Tables 1 and 3) with those of **1** suggested that **1** and **2** differed from each other only in the rings A and B′. Apart from signals for rings CDE, signals for a 1-oxygen-1-en-3-one system (δ 198.3, C-3; 187.1, C-1; 97.0, C-2), one ester (δ 173.2), two methyls (δ 27.8, C-28; 19.9, C-19), two sp^3 methylenes (δ 72.2, C-29; 30.5, C-6), an sp^3 methine (δ 46.5, C-5), and two sp^3 quaternary carbons (δ 44.2, C-4; 48.4, C-10) were also observed in the ^{13}C NMR spectrum of **2**. The above suggested that **2** possessed an intact 1-oxygen-1-en-3-one ring A and a possible six-membered lactone formed between the ester carbonyl C-7 and one oxymethylene attributable to C-19, C-28, or C-29. The proposed structure and the location of the oxymethylene were further determined by the HMBC experiments (Figure 2). HMBC correlations from the oxymethylene to C-3, C-4, C-5, and C-7 indicated that the oxymethylene was assignable to C-28 or C-29 to form a six-membered 7,28- or 7,29-lactone ring. A NOESY correlation (Figure 2) observed between H_3-19 and H-29β ruled out the 7,28-lactone ring and indicated the 7,29-lactone in **2**. The 1-oxygen-1-en-3-one functionality was confirmed by correlations of H-2 to C-1, C-4, and C-10 in the HMBC spectrum. A proton singlet at δ_H 6.98, which showed no correlations in the HMQC spectrum but correlated with C-8 and C-9 in the HMBC spectrum, was assigned to OH-8. The stereochemistry of **2** was established in the same way as that of **1**. The broad singlet of H-12 and the NOESY correlation between H-12 and H-17 were also consistent with a 12α-OH in **2**. The structure of **2** was therefore established.

The molecular formula $C_{29}H_{36}O_{10}$ was given to toonaciliatin C (**3**) on the basis of its HREIMS. Its UV spectrum was very similar to those of **1** and **2**. The ^1H NMR spectrum of **3** was very close to that of **2**, except for additional signals for acetoxy (δ_H 2.05) and methoxy (δ_H 3.71) groups. Their ^{13}C NMR data (Table 3) suggested that both compounds shared the same ACDE ring system. Nevertheless, the carbon signal for the 29-oxymethylene was upfield shifted to δ 66.3 by comparison with **2**, indicating that the 7,29-lactone in **2** was opened in **3**. The acetyloxy was thus likely to be located at C-29 and the methoxy at C-7. HMBC correlations from H_2-29 to C-3, C-4, C-5, C-28, and the acetyl carbonyl, as well as the NOESY correlation between H_2-29 and H_3-19, confirmed the location of the acetyloxy group at C-29. HMBC correlations from the OMe to C-7 attached the methoxy to C-7. Further analysis of the HMBC and NOESY spectra confirmed the structure of **3**.

Table 1. ^{1}H NMR Data (δ_H, mult, J_{HH} in Hz) of Toonaciliatins A−D (**1**−**4**) (400 MHz)

proton	**1** (CD_3OD)	**2** (C_5D_5N)	**3** ($CDCl_3$)	**4** ($CDCl_3$)
1				4.44, br d (7.6)
2a				2.32, dd (15.7, 7.6)
2/2b	5.40, s	5.58, s	5.43, s	3.58, dd (15.7, 1.0)
5		3.45, dd (6.6, 6.9)	2.78, d (10.1)	1.90, d (13.8)
6α/6a	4.89, s	3.48, dd (16.4, 6.9)	2.72, dd (14.2, 10.1)	2.59, d (12.2)
6β/6b		3.42, dd (16.7, 6.6)	3.12, d (14.2)	2.96, dd (13.8, 12.2)
9	4.11, d (12.4)	3.40, d (12.4)	2.91, d (12.2)	2.59, d (12.2)
11β	4.82, dd (12.4, 4.8)	5.09, dd (12.4, 4.7)	4.73, dd (12.2, 4.7)	4.17, ddd (12.2, 8.3, 7.7)
12α				1.60, dd (13.1, 8.3)
12β	4.43, d (4.8)	4.78, brs	4.43, d (4.7)	2.38, dd (13.1, 7.7)
15	3.61, s	3.69, s	3.60, s	3.61, s
16α	1.94, dd (13.4, 11.0)	1.91, dd (13.4, 11.3)	1.86, dd (13.5, 11.1)	1.85, dd (13.5, 11.2)
16β	2.24, dd (13.4, 6.6)	2.26, dd (13.4, 6.6)	2.35, dd (13.5, 6.6)	2.24, dd (13.5, 6.4)
17	2.83, dd (11.0, 6.6)	3.08, dd (10.9, 6.6)	2.85, dd (11.1, 6.6)	2.74, dd (11.2, 6.4)
18	1.05, s (3H)	1.58, s (3H)	1.05, s (3H)	1.00, s (3H)
19	1.46, s (3H)	1.54, s (3H)	1.41, s (3H)	1.24, s (3H)
21	7.29, dd (1.4, 1.0)	7.51, br s	7.21, br s	7.12, br s
22	6.39, dd (2.0, 1.0)	7.17, d (1.3)	6.35, br s	6.17, br s
23	7.41, dd (2.0, 1.4)	7.61, dd (1.8, 1.3)	7.41, dd (1.6, 1.4)	7.37, br s
28	1.55, s (3H)	1.18, s (3H)	1.05, s (3H)	1.15, s (3H)
29/29α/29a		4.44, d (11.5)	4.21, d (11.8)	1.41, s (3H)
29β/29b		4.74, d (11.5)	4.41, d (11.8)	
30	1.27, s (3H)	1.58, s (3H)	1.26, s (3H)	1.41, s (3H)
8-OH		6.98, s		
29-OAc			2.05, s (3H)	
7/3-OMe			3.71, s (3H)	3.68, s (3H)

Table 2. ^{1}H NMR Data (δ_H, mult, J_{HH} in Hz) of Toonaciliatins E−G (**5**−**7**) (400 MHz)

proton	**5** ($CDCl_3$)	**6** (CD_3OD)	**7** (CD_3OD)
1	6.07, d (13.0)	4.10, dd (11.5, 6.6)	4.11, dd (11.4, 6.5)
2α		2.14, ddd (13.6, 13.2, 11.5)	1.59, ddd (13.3, 13.1, 11.4)
2/2β	6.74, d (13.0)	1.93, ddd (13.6, 6.6, 4.5),	2.04, ddd (13.3, 6.5, 4.7)
3		4.41, dd (13.2, 4.5)	4.40, dd (13.1, 4.7)
5	3.57, s		
6/6α	5.42, s	3.89, s	3.89, s
9	2.06, br s	3.05, d (12.0)	3.03, d (12.3)
11α	1.93, m		
11β	1.65, m	4.12, dd (12.0, 6.2)	4.20, ddd (12.3, 9.8, 7.2)
12α	0.97, ddd (13.1, 6.3, 2.6)		1.53, dd (12.6, 9.8)
12β	1.57, m	4.09, d (6.2)	2.17, dd (12.8, 7.2)
15	4.30, d (5.5)	3.57, s	3.59, s
16α	2.13, td (13.5, 5.5)	1.88, dd (13.5, 10.9)	1.88, dd (13.2, 11.0)
16β	1.79, dd (13.5, 5.5)	2.21, dd (13.5, 7.1)	2.17, dd (13.2, 6.4)
17	3.24, dd (13.5, 5.5)	2.75, dd (10.9, 7.1)	2.71, dd (11.0, 6.4)
18	0.69, s (3H)	0.98, s (3H)	1.06, s (3H)
19	1.42, s (3H)	1.47, s (3H)	1.45, s (3H)
21	7.25, dd (1.6, 1.0)	7.26, d (1.0)	7.27, d (1.3)
22	6.26, dd (1.7, 1.0)	6.41, d (1.5)	6.28, d (1.7)
23	7.34, dd (1.7, 1.6)	7.39, dd (1.5, 1.0)	7.43, dd (1.7, 1.3)
28	1.51, s (3H)	1.47, s (3H)	1.49, s (3H)
29	1.73, s (3H)		
30	1.76, s (3H)	1.25, s (3H)	1.25, s (3H)
8-OH		4.59, s	4.60, s
6-OAc	2.19, s (3H)		
7-OMe	3.77, s (3H)		

The HREIMS peak at *m*/*z* 488.2424 (calcd 488.2410) indicated that **4** had the molecular formula of $C_{27}H_{36}O_8$. Carbon signals assignable to a methoxy and a typical limonoid core comprising 26 carbons were resolved in the ^{13}C NMR spectrum. Comparison of the ^{1}H and ^{13}C NMR data of **4** with those of toonafolin,[20] a B-*seco* limonoid isolated from *Toona ciliata*. M. J. Roem. var. *australis*, indicated that their structures were closely related, with the major difference being the absence of the C-3 carbonyl (δ_C 213.2) and C-4 quaternary carbon (δ_C 45.6) and the presence of an additional ester (δ_C 173.8), an extra oxygenated quaternary carbon (δ_C 74.4), and a methoxy (δ_C 51.8) in **4**. These observations suggested that **4** was possibly an A-*seco* product of toonafolin via oxidation and was likely to have a methoxycarbonyl at C-3 and a hydroxyl at C-4. HMBC experiments (Figure 3) confirmed the proposal. NOESY correlations (Figure 3) were then used to establish the stereochemistry of **4**. Despite the fact that the stereochemsitry of the BCDE ring system was the same as that of toonafolin, the NOESY correlation between H-1 and H-9 suggested that H-1 was on the α-side of the tetrahydrofuran ring, which was different from that of toonafolin. The structure of **4** was therefore established.

Toonaciliatin E (**5**) was determined to have the molecular formula $C_{29}H_{38}O_9$ by HRESIMS, which was supported by positive-mode ESIMS peaks at *m*/*z* 553.2 $[M + Na]^+$ and 1084.3 $[2M + Na]^+$ as well as the negative-mode ESIMS peak at *m*/*z* 575.4 $[M + HCOOH - H]^-$. However, except for signals for an acetoxyl and a methoxy, only 24 carbon signals in the limonoid core were observed in the ^{13}C NMR spectrum of **5** (Table 3). Extensive 2D NMR experiments (HSQC, HMBC, and ROESY) were thus carried out to reveal the structure for **5**. The HSQC correlations from both signals at δ_H 3.57 (H-5) and 2.06 (H-9) to a carbon signal at δ_C 51.6 (a methine signal by the DEPT spectrum) indicated that it was two overlapped methines, which were then assigned to C-5 and C-9 on the basis

Table 3. ^{13}C NMR Data of Toonaciliatins A−G (**1**−**7**) and **8** (100 MHz)

position	**1**[a]	**2**[b]	**3**[c]	**4** *c*	**5** *c*	**6**[a]	**7**[a]	**8**[a]
1	191.8	187.1	185.7	87.0	150.7	87.1	86.7	87.4
2	97.7	97.0	99.0	38.5	121.4	36.9	38.8	49.7
3	193.8	198.3	199.4	173.8	166.5	71.4	71.4	206.3
4	86.3	44.2	48.4	74.4	83.7	95.8	95.6	92.5
5	82.0	46.5	48.4	57.0	51.6	83.9	84.0	85.5
6	77.3	30.5	32.9	37.6	70.1	77.8	77.7	76.1
7	174.9	173.2	174.0	173.0	170.3	178.0	177.8	175.4
8	77.3	74.3	75.1	82.3	65.9	76.5	76.5	76.9
9	47.0	55.3	54.2	61.8	51.6	50.3	55.1	50.8
10	53.6	48.4	49.2	50.6	46.7	52.8	52.9	52.8
11	85.3	83.3	80.9	71.9	20.0	79.0	75.6	79.7
12	73.4	71.0	71.0	42.4	29.2	73.3	48.9	73.0
13	48.6	47.6	46.5	41.3	41.8	48.4	43.5	48.5
14	74.3	74.0	73.3	71.6	77.2[d]	74.6	74.6	74.4
15	57.2	56.3	55.7	56.1	74.9	57.2	57.4	57.3
16	32.8	31.6	31.0	31.0	39.0	32.7	32.9	32.7
17	43.6	41.6	41.1	41.8	42.6	43.5	43.2	43.2
18	16.1	16.4	15.4	23.0	18.5	16.3	24.2	16.4
19	23.3	19.9	19.6	12.8	24.3	19.2	18.8	19.3
20	124.7	123.0	122.2	122.5	123.1	124.9	124.6	124.8
21	141.4	140.2	139.4	139.4	139.7	141.3	141.4	141.3
22	112.7	111.8	110.6	110.5	110.8	112.7	112.2	112.6
23	144.4	143.3	143.2	143.1	142.6	144.4	144.8	144.4
28	22.6	27.8	25.3	24.7	25.6	15.5	15.8	20.9
29		72.2	66.3	34.1	30.1			
30	23.3	24.2	23.5	20.1	27.0	23.5	23.3	23.6
6/29-OAc			170.4		169.5			
			20.9		21.0			
7/3-OMe			52.2	51.8	53.2			

[a] Recorded in CD_3OD. [b]Recorded in C_5D_5N. [c]Recorded in $CDCl_3$. [d]Buried in signals of $CDCl_3$.

of HMBC correlations from both H-6 and H-12 to this carbon signal. An oxygenated quaternary carbon signal buried under the signals of deuterated chloroform in the ^{13}C NMR spectrum was revealed to be C-14 by the correlations with H_3-18, H-15, and H_3-30 in the HMBC spectrum (Figure 4). Twenty-six skeletal carbon signals were therefore recognized, and extensive HMBC analysis further established the structure **5**. In particular, a ^4J HMBC correlation from H_3-28 to C-3 constructed a seven-membered 3,4-lactone ring, and HMBC correlations from H-6 to the acetyl carbonyl and from OMe to the C-7 carbonyl located the acetoxyl and methoxy at C-6 and C-7, respectively. The oxygenated quaternary carbon signal at δ_C 65.9 suggested the presence of an epoxide ring in **5**, but the HMBC correlations from H-15 to C-16 and C-17 ruled out a 14,15-epoxide ring. HMBC correlations from H_3-30 to C-8 (δ_C 65.9) and C-14 (δ_C 77.2) and from H_3-18 to C-14 established an unusual 8,14-epoxide ring. The oxygen function at C-15 was therefore only assignable to a free hydroxyl as deduced from its molecular formula. The planar structure of **5** constructed by extensive HMBC analysis closely resembled that of **9**,[15] a semisynthetic 8,14-epoxide-containing limonoid also isolated in this investigation as a natural compound for the first time. The only difference between compounds **5** and **9** is the presence of an acetoxyl at C-6 in **5**. In the ROESY spectrum (Figure 4), the correlation between H-15 and H_3-18 was indicative of the presence of a H-15α, thus placing the OH-15 in the β-configuration. ROESY correlations of H_3-19/H_3-30, H-2/H-17, H-12β/H-17, and H-12β/H-16β suggested that the 8,14-epoxide ring was in the α-orientation. The singlet of H-6 suggested that the dihedral angle between H-6 and H-5 is ca. 90° in its preferred conformation, and the observed ROESY correlations of OMe/H_3-28, OAc/H_3-19, and OAc/H_3-29 were consistent with a 6*R* configuration, as indicated in its Newman projection. The structure of **5** was thus fully defined.

Compound **6** gave a molecular formula of $C_{25}H_{32}O_{10}$ (HREIMS). Proton signals characteristic for a β-substituted furan ring and four tertiary methyl groups were evident (Tables 2 and 3), indicating that **6** was a norlimonoid. Its ^{1}H and ^{13}C NMR data were very similar to those of compound **8**. The main difference was the replacement of the C-3 ketone (δ_C 206.3) of **8** with an oxymethine at δ_C 71.4 in **6**, suggesting that **6** was a 3-OH derivative of **8**. HMBC correlations from H-3 to C-2, C-4, and C-28 confirmed the presence of a 3-OH group, while NOESY correlations from H-1 to both H-3 and H_3-19 placed the 3-OH in the α-configuration. Comprehensive 2D NMR (HMQC, HMBC, and NOESY) correlation analysis further established the full structure **6** for toonaciliatin F.

Toonaciliatin G (**7**) had a molecular formula of $C_{25}H_{32}O_9$, as indicated by HREIMS. Comparison of the ^{1}H and ^{13}C NMR data of **7** (Tables 2 and 3) with those of **6** indicated that **7** differed from **6** only at C-12, where a methylene (δ_C 48.9) of **7** had replaced the oxymethine (δ_C 73.3) of **6**. Extensive 2D NMR (HMQC, HMBC, and NOESY) correlation analysis confirmed this assumption, and the structure of **7** was thus established.

The known compounds 5α,6β,8α-trihydroxy-28-norisotoonafolin (**8**),[18] toonaciliatin H (**9**),[15] toonaciliatin I (**10**),[15] febrifugin (**11**),[21] and khayasin T (**12**)[22] were identified by comparison of their spectroscopic data with those reported in the literature.

The co-occurrence of **5**, **9**, and **10** in the same source suggested that limonoids **5** and **9** were possibly derived from **10** through an acid-catalyzed intramolecular rearrangement, and a subsequent oxidation and acetylation of **9** produced **5**. Although the chemical transformation of **10** into **9** was conducted in methanolic potassium hydroxide and through a very different mechanism,[15] Lewis acid (boron trifluoride)-catalyzed intramolecular rearrangement of epoxy alcohols with similar stereoselectivity has been reported.[23]

Based on the co-occurrence of the 12 limonoids and norlimonoids in the same origin and the possible mechanisms of conversion, a biogenetic map that encompassed the pathways for all limonoids, and started from the common precursor (**i**) was proposed (Scheme S1, Supporting Information). The limonoids and norlimonoids appear to be formed through a variety of pathways from a common precursor, which supports the earlier chemosystematic conclusion that *Toona* would preferably be included in the Melioideae rather than the Swietenioideae subfamily.[16−18,24] Yet, the abundant occurrence of two mexicanolide-type limonoids (**11** and **12**), which occur widely in the genera of the Swietenioideae subfamily, suggests that the affiliation of *Toona* to the Melioideae was also

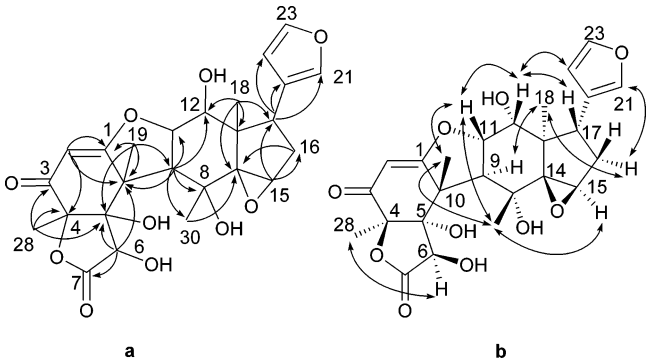


Figure 1. Selected HMBC (H→C) (a) and NOESY (↔) (b) correlations of toonaciliatin A (**1**).

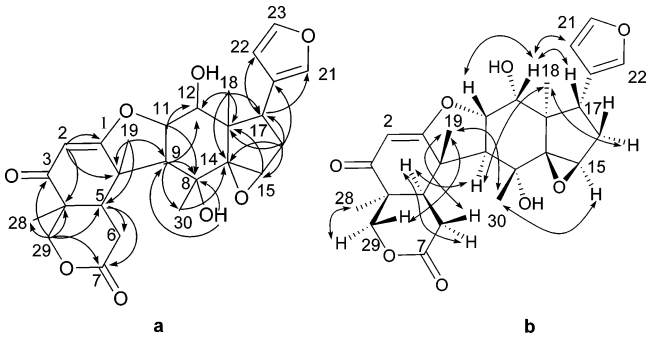


Figure 2. Selected HMBC (H→C) (a) and NOESY (↔) (b) correlations of toonaciliatin B (**2**).

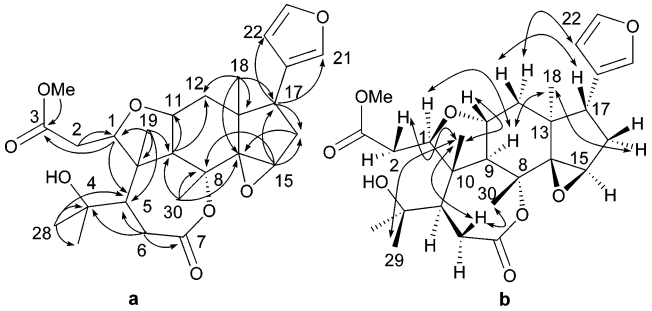


Figure 3. Selected HMBC (H→C) (a) and NOESY (↔) (b) correlations of toonaciliatin D (**4**).

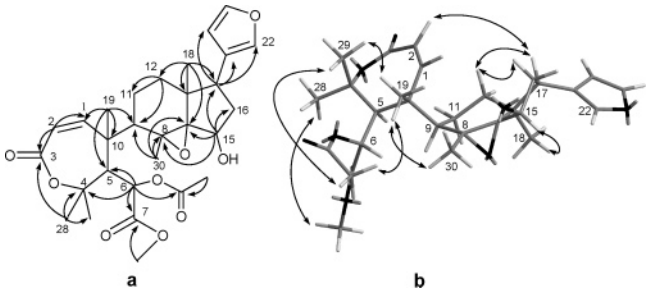


Figure 4. Selected HMBC (H→C) (a) and NOESY (↔) (b) correlations of toonaciliatin E (**5**).

problematic. *Toona* was therefore most likely to be peripheral Melioideae as well as Swietenioideae,[2] and it might be more reasonable to treat this genus as a separate subfamily.

Experimental Section

General Experimental Procedures. The following instruments were used to obtain physical data: Optical rotations were determined on a Perkin-Elmer 341 polarimeter, and CD spectra were obtained on a JASCO 810 spectrometer. UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer with KBr disks. NMR spectra were measured on a Bruker AM-400 spectrometer. EIMS and HREIMS (70 eV) were carried out on a Finnigan MAT 95 mass spectrometer, and ESIMS was carried out on a Finnigan LC QDECA instrument. The following experimental materials were used for chromatography: silica gel (200−300 mesh) and silica gel H (Qingdao Haiyang Chemical Co. Ltd.); C18 reversed-phase silica gel (150−200 mesh, Merck); MCI gel (CHP20P, 75−150 μm, Mitsubishi Chemical Industries Ltd.); Sephadex LH-20 gel (Amersham Biosciences). TLC plates were precoated with silica gel GF254 (Merck, 0.25 mm) (ordinary phase), and detection was achieved by spraying with 10% H_2SO_4 in ethanol followed by heating. All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd.).

Plant Material. The leaves and stems of *Toona ciliata* were collected in Xishuangbanna Tropical Botanical Garden, Mengla County, Yunnan Province, in February 2005. The plant was identified by one of the authors (Y.-K.X.). A voucher specimen has been deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (accession number Tc-2005-1YL).

Extraction and Isolation. A powder of the dried leaves and stems of *T. ciliata* (4.6 kg) was extracted three times with 95% EtOH under reflux. Evaporation of the solvent under reduced pressure provided the EtOH extract (265 g), which was then extracted with EtOAc. Fractionation of the EtOAc-soluble fraction (95 g) with an MCI column (MeOH/H_2O, 40/60 → 90/10, v/v) gave fractions 1−3. Fraction 2 obtained from the 50−80% MeOH in H_2O was subjected to silica gel column chromatography using a gradient solvent system of petroleum ether/acetone (20/1 to 0/1; v/v) to give three major fractions, 2a−2c. Fraction 2a (1.51 g) was fractionated by reversed-phase silica gel column chromatography (CC) (MeOH/H_2O, 40/60 → 70/30, v/v) to afford six fractions, 2a.1−2a.6. Separation of fraction 2a.2 (0.115 g) by silica gel column chromatography (petroleum ether/EtOAc, 3/1 → 2/1, v/v) afforded subfractions 2a.2.1−2a.2.2. Purification of fraction 2a.2.2 by silica gel CC ($CHCl_3$/acetone, 40/1, v/v) gave **10** (9 mg). Chromatographic separation of fraction 2a.3 (109 mg) on a silica gel column (petroleum ether/2-propanol, 10/1 → 8/1, v/v) gave two subfractions, purification of which with reversed-phase silica gel CC (MeOH/H_2O, 60/40, v/v) furnished **4** (5 mg) and **9** (8 mg), respectively. Fraction 2a.4 (332 mg) was subjected to silica gel CC ($CHCl_3$/acetone, 40/1, v/v) to give two subfractions, 2a.4.1 and 2a.4.2. Purification of the latter with $CHCl_3$/acetone (40/1, v/v) yielded **5** (12 mg). Fraction 2b was subjected to reversed-phase silica gel CC (MeOH/H_2O, 40/60 → 70/30, v/v) to afford four fractions, 2b.1−2b.4. Separation of fraction 2b.1 and 2b.3 with silica gel CC ($CHCl_3$/MeOH, 30/1 and 15/1, respectively, v/v) gave **3** (8 mg) and **6** (10 mg), respectively, while that of fraction 2b.2 ($CHCl_3$/MeOH, 30/1 → 20/1, v/v) yielded **2** (12 mg) and **7** (9 mg). Fraction 2c was subjected to reversed-phase silica gel CC (MeOH/H_2O, 40/60 → 70/30, v/v) to afford three fractions, 2c.1−2c.3. Purification of fractions 2c.1 and 2c.3 with Sephadex LH-20 (MeOH) furnished **8** (61 mg) and **1** (10 mg), respectively. Silica gel CC of fraction 3 afforded fractions 3a−3c. Fraction 3a was then subjected to reversed-phase silica gel CC (MeOH/H_2O, 75/25 → 85/15, v/v) to afford four fractions, 3a.1−3a.4. Purification of fraction 3a.4 with silica gel CC ($CHCl_3$/MeOH, 100/1, v/v) afforded **11** (510 mg). Reversed-phase silica gel CC (MeOH/H_2O, 75/25 → 85/15, v/v) of fraction 3c yielded two fractions, 3c.1 and 3c.2, and purification of the former by silica gel CC (petroleum ether/EtOAc, 4/1, v/v) furnished **12** (77 mg).

Toonaciliatin A (1): white powder; $[\alpha]^{21}_D$ +91 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.48), 268 (4.38) nm; IR (KBr) γ_{max} 3483, 2928, 1782, 1628, 1383, 1153, 1061, 959, 602 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 3; positive-ion ESIMS *m*/*z* 489.0 $[M + H]^+$, 999.2 $[2M + Na]^+$; negative-ion ESIMS *m*/*z* 487.2 $[M - H]^-$, 975.7 $[2M - H]^-$; EIMS *m*/*z* 488 (1), 470 (10), 452 (12), 409 (8), 253 (24), 189 (36), 175 (68), 151 (100), 122 (68); HREIMS *m*/*z* 488.1700 (calcd for $C_{25}H_{28}O_{10}$ M^+, 488.1682).

Toonaciliatin B (2): white powder; $[\alpha]^{21}_D$ −21 (*c* 0.2, MeOH); CD (MeOH) $\Delta\epsilon$ (λ_{max}) −13.6 (300), +22.0 (259), −1.0 (205), −0.2 (197); UV (MeOH) λ_{max} (log ϵ) 212 (3.61), 259 (4.46) nm; IR (KBr) γ_{max} 3450, 2982, 2939, 1738, 1622, 1390, 1205, 1151, 1057, 1013, 959, 598 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz), see Table 3; positive-ion ESIMS *m*/*z* 471.1

$[M + H]^+$, 963.3 $[2M + Na]^+$; negative-ion ESIMS *m/z* 515.3 $[M + HCOOH - H]^-$, 939.2 $[2M - H]^-$; EIMS *m/z* 470 (1), 452 (7), 434 (7), 189 (29), 175 (39), 151 (100), 122 (63); HREIMS *m/z* 470.1962 (calcd for $C_{26}H_{30}O_8$ M^+, 470.1941).

Toonaciliatin C (3): white powder; $[\alpha]^{21}_D$ +60 (*c* 0.22, MeOH); CD (MeOH) $\Delta\epsilon$ (λ_{max}) −9.9 (301), +23.3 (260), −0.8 (207), −0.06 (198); UV (MeOH) λ_{max} (log ϵ) 209 (3.62), 259 (4.18) nm; IR (KBr) γ_{max} 3462, 2929, 1732, 1643, 1435, 1375, 1252, 1202, 1034, 959, 598 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; positive-ion ESIMS *m/z* 545.1 $[M + H]^+$, 1111.3 $[2M + Na]^+$; negative-ion ESIMS *m/z* 589.2 $[M + HCOOH - H]^-$, 1087.2 $[2M - H]^-$; EIMS *m/z* 544 (2), 526 (5), 508 (4), 486 (4), 485 (15), 358 (15), 189 (38), 175 (14), 151 (100), 122 (56); HREIMS *m/z* 544.2290 (calcd for $C_{29}H_{36}O_{10}$ M^+, 544.2308).

Toonaciliatin D (4): white powder; $[\alpha]^{21}_D$ +10 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (3.67) nm; IR (KBr) γ_{max} 3450, 2920, 2850, 1728, 1441, 1385, 1173, 1065, 995, 602 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; positive-ion ESIMS *m/z* 999.4 $[2M + Na]^+$; negative-ion ESIMS *m/z* 533.3 $[M + HCOOH - H]^-$; EIMS *m/z* 488 (2), 473 (9), 441 (12), 415 (12), 399 (25), 355 (52), 189 (87), 155 (77), 121 (87), 95 (100); HREIMS *m/z* 488.2424 (calcd for $C_{27}H_{36}O_8$ M^+, 488.2410).

Toonaciliatin E (5): white powder; $[\alpha]^{21}_D$ −41 (*c* 0.14, MeOH); CD (MeOH) $\Delta\epsilon$ (λ_{max}) +7.0 (253), +2.9 (223), +3.7 (219), −8.0 (193); UV (MeOH) λ_{max} (log ϵ) 215 (4.18) nm; IR (KBr) γ_{max} 3429, 2922, 1738, 1637, 1375, 1219, 1126 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 3; positive-ion ESIMS *m/z* 553.2 $[M + Na]^+$, 1084.3 $[2M + Na]^+$; negative-ion ESIMS *m/z* 575.4 $[M + HCOOH - H]^-$; EIMS *m/z* 530 (28), 247 (16), 229 (59), 224 (100), 159 (63); HREIMS *m/z* 530.2519 (calcd for $C_{29}H_{38}O_9$ M^+, 530.2516).

Toonaciliatin F (6): white powder; $[\alpha]^{21}_D$ +92 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (3.61) nm; IR (KBr) γ_{max} 3415, 2953, 1763, 1632, 1448, 1387, 1273, 1070, 1013, 949, 874, 598 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 2; ^{13}C NMR (CD_3OD, 100 MHz), see Table 3; positive-ion ESIMS *m/z* 515.1 $[M + Na]^+$, 1007.2 $[2M + Na]^+$; negative-ion ESIMS *m/z* 491.1 $[M - H]^-$, 983.4 $[2M - H]^-$; EIMS *m/z* 474 (11), 456 (56), 441 (10), 413 (18), 295 (22), 189 (52), 175 (100), 147 (67); HREIMS *m/z* 492.1995 (calcd for $C_{25}H_{32}O_{10}$ M^+, 492.1995), 474.1900 (calcd for $C_{25}H_{30}O_9$ $[M - H_2O]^+$, 474.1890).

Toonaciliatin G (7): white powder; $[\alpha]^{21}_D$ +61 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (3.83) nm; IR (KBr) γ_{max} 3332, 2937, 1755, 1740, 1460, 1390, 1277, 1082, 1014, 797, 600 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 2; ^{13}C NMR (CD_3OD, 100 MHz), see Table 3; positive-ion ESIMS *m/z* 459.1 $[M + H - H_2O]^+$, 975.3 $[2M + Na]^+$; negative-ion ESIMS *m/z* 475.2 $[M - H]^-$, 951.6 $[2M - H]^-$; EIMS *m/z* 476 (2), 458 (10), 443 (48), 425 (22), 297 (39), 239 (31), 189 (50), 160 (62); HREIMS *m/z* 476.2054 (calcd for $C_{25}H_{32}O_9$ M^+, 476.2046).

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Supporting Information Available: 2D NMR spectra of toonaciliatins A−G (**1**−**7**). This material is available free of charge via the Internet at http://pubs.acs.org.

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