Terpenoids from *Toona ciliata*

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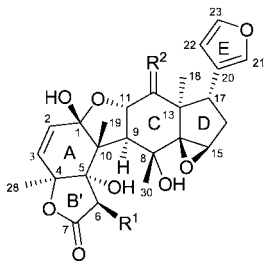
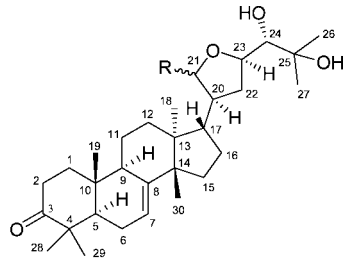
Three new norlimonoids (**1**−**3**), two new tirucallane-type triterpenoids (**4** and **5**), and a new pimaradiene-type diterpenoid (**6**), along with two known limonoids and eight known tirucallane-type triterpenoids, were isolated from the leaves and twigs of *Toona ciliata*. Their structures were elucidated on the basis of spectroscopic data. Toonaciliatin M (**6**) showed moderate antifungal activity against *Trichophyton rubrum* with an MIC of 12.5 μg/mL.

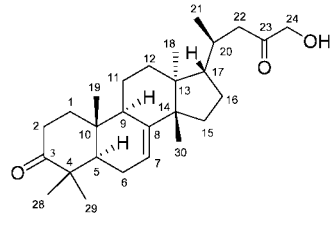
Plants of the Meliaceae family are rich sources of structurally diverse and biologically significant limonoids.[1] The plant of *Toona ciliata* Roem. var. *ciliata* (Meliaceae) is a timber tree mainly growing in the tropical areas of Asia such as India, Malaysia, Indonesia, and southern China.[2] The bark has been applied to treat dysentery, fever, and menstrual disorders in Chinese folk medicine.[3] Previous chemical investigations on *T. ciliata* have led to the isolation of a series of limonoids,[4] norlimonoids,[5] and coumarins.[6] As a part of our continuous studies on the Meliaceae family,[7] three new norlimonoids (**1**−**3**), two new tirucallane-type triterpenoids (**4** and **5**), and a new pimaradiene-type diterpenoid (**6**), along with 10 known compounds, were isolated from the EtOH extract of the leaves and twigs of *T. ciliata* Roem.var. *ciliata*, which was collected from the Hainan Province of China. Their structures were elucidated on the basis of spectroscopic data. We report herein the isolation and structural elucidation of these new compounds, along with the antimicrobial evaluation of all the isolates against 11 microbes *in vitro*.



1 R^1 = OAc, R^2 = α-OH
2 R^1 = OAc, R^2 = O
3 R^1 = OH, R^2 = α-OH



4 R = β-OEt
4a R = α-OEt



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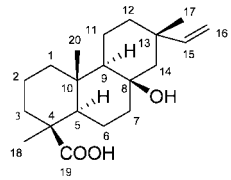

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Results and Discussion

Toonaciliatin H (**1**) was obtained as a white, amorphous powder. Its molecular formula was determined as $C_{27}H_{32}O_{11}$ by HRESIMS, indicating an *m/z* ion at 555.1824 [M + Na]$^+$ (calcd for $C_{27}H_{32}O_{11}Na$, 555.1842). IR absorptions showed the presence of hydroxy (3442 cm^{-1}) and carbonyl (1776 cm^{-1}) groups. The ^{13}C NMR spectrum resolved 27 carbon resonances, which were classified by chemical shifts and the HSQC spectrum as five methyls, one methylene, 11 methines (four oxygenated and five olefinic), and 10 quaternary carbons (two ester carbonyls, one olefinic, one hemiketal, and four oxygenated carbons). These functionalities occupied five out of the 12 degrees of unsaturation; the remaining seven double-bond equivalents therefore required compound **1** to possess seven rings. In addition, the presence of one acetyl (δ_H 2.20, 3H, s), four tertiary methyls (δ_H 1.45, 1.65, 1.82, and 2.04, each 3H, s), and one β-substituted furan ring (δ_H 6.68, 7.46, and 7.61) was distinguished by analysis of its ^1H NMR data (Table 1). The aforementioned data indicated that compound **1** featured a norlimonoid skeleton sharing similar B′CDE rings of 5α,6β,8α-trihydroxy-28-norisotoonafolin,[5b] which was also isolated in this investigation.

A detailed account of the structural assignment of **1** is given as below. The β-furan ring was attached to C-17 by the HMBC correlations (see Supporting Information) of H-17/C-20, C-21, and C-22. The presence of a 14,15-epoxide moiety was revealed by the chemical shifts of C-14 at δ_C 73.1 and C-15 at δ_C 55.9 and was confirmed by the mutual HMBC correlations from H-15 to C-14, C-16, and C-17 and from Me-30, H_2-16, and H-12 to C-14. Three hydroxy protons resonated at δ 6.83, 8.28, and 5.55 and were located at C-5, C-8, and C-12 on the basis of the HMBC correlations of OH-5/C-5(δ_C 82.1), OH-8/C-8 (δ_C 76.1), and OH-12/C-12 (δ_C 71.8), respectively. The five-membered lactone B′-ring was established by comparing the chemical shifts of C-7 (δ_C 171.2) and C-4 (δ_C 88.1) with those of a coexisting limonoid, 5α,6β,8α-trihydroxy-28-norisotoonafolin.[5b] The only *O*-acetyl group was located at C-6 by the HMBC correlation from H-6 to its carbonyl at δ_C 169.5, which caused C-7 to shift upfield by about 3.5 ppm due to the γ-gauche effect of OAc-6. A Δ^2 double bond was located on the basis of the carbon resonances of C-2 and C-3 (also the proton resonances of H-2 and H-3) (Table 1) and confirmed by the HMBC correlations of H-2/C-1, C-4, and C-10 and H-3/C-5. The chemical shifts of C-1 at δ_C 106.7 and C-11 at δ_C 75.9 indicated that a hemiketal was formed at C-1 via an oxygen bridge between C-1 and C-11, which was confirmed by the HMBC correlations from OH-1 (δ_H 8.57) to C-1, C-2, and C-10.

The relative configuration of **1** was established by a ROESY experiment (see Supporting Information). The ROESY correlations of Me-18/H-9, Me-18/H-16α, Me-18/Me-28, H-9/Me-28, H-9/OH-5, and H-6/OH-5 indicated that Me-18, Me-28, H-9, OH-5, and H-6 were cofacial and were randomly assigned to be α-oriented. Subsequently, the ROESY correlations of Me-19/H-11, Me-19/Me-30, Me-30/H-11, H-11/H-12, H-12/H-17, and H-17/H-16β indicated that they were cofacial and β-oriented. The 14,15-epoxide ring was tentatively assigned as β-oriented by comparison of the relevant

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**3**[a] in C_5D_5N

	1		**2**		**3**	
no.	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C
1		106.7		106.1		106.4
2	6.24, d (10.5)	132.9	6.21, d (10.4)	132.9	6.27, d (10.5)	133.3
3	5.95, d (10.5)	125.8	5.85, d (10.4)	125.5	5.94, d (10.5)	126.3
4		88.1		87.5		87.2
5		82.1		82.0		82.1
6	6.10, (s)	75.5	6.09, s	75.3	4.71, s	77.9
7		171.2		170.7		174.3
8		76.1		75.5		76.0
9	3.71, d (12.1)	50.3	3.73, d (13.7)	55.2	3.80, d (12.1)	49.7
10		54.6		54.7		55.2
11	4.74, dd (12.1, 4.1)	75.9	5.17, d (13.7)	78.5	4.86, dd (12.1, 4.7)	77.4
12	4.46, d (4.1)	71.8		210.2	4.51, d (4.7)	71.9
13		46.6		53.9		46.9
14		73.1		72.1		73.1
15	3.59, s	55.9	3.77, s	57.3	3.61, s	55.8
16α	1.88, dd (13.2, 10.8)	31.3	1.98, dd (13.5, 11.0)	31.4	1.88, dd (13.5, 10.5)	31.4
16*β*	2.23, dd (13.2, 6.7)		2.21, dd (13.5, 7.0)		2.24, dd (13.5, 6.4)	
17	2.99, dd (10.8, 6.7)	41.9	3.17, dd (11.0, 7.0)	37.9	3.01, dd (10.5, 6.4)	42.0
18	1.45, s	15.9	1.45, s	18.9	1.47, s	15.9
19	1.82, s	13.8	1.81, s	13.2	2.02, s	13.4
20		124.1		123.5		124.1
21	7.46, s	140.0	7.53, s	141.5	7.46, s	140.0
22	6.68, s	111.7	6.90, s	113.1	6.71, s	111.8
23	7.61, s	143.2	7.60, s	143.1	7.61, s	143.2
28	2.04, s	24.1	1.91, s	24.1	2.02, s	24.3
30	1.65, s	24.1	1.73, s	23.6	1.71, s	24.1
−OAc	2.20, s	21.2, 169.5	2.20, s	21.2, 169.5		
1-OH	8.57, brs		9.04, brs			
5-OH	6.83, brs		6.84, brs		6.33, brs	
8-OH	8.28, brs		8.49, brs		8.09, brs	
12-OH	5.55, brs					

[a] Data were recorded at 400 and 100 MHz for ^{1}H and ^{13}C, respectively.

NMR data of **1** with those of toonaciliatin B,[5b] including the chemical shifts of protons and carbons as well as coupling patterns of the protons around the epoxide. This was verified by the ROESY correlation between H-15α and OH-8. C-19 at δ 13.8 of **1** was severely upfield shifted ($\Delta\delta$ ca. 6.1) as compared with that of 5α,6*β*,8α-trihydroxy-28-norisotoonafolin,[5b] indicting that OH-1 and Me-19 were in a γ-gauche relationship, and OH-1 was therefore *β*-oriented. The structure of **1** was thus fully assigned as depicted.

Toonaciliatin I (**2**), a white, amorphous powder, was assigned a molecular formula of $C_{27}H_{30}O_{11}$ as determined by HRESIMS, indicating an *m*/*z* ion at 553.1666 $[M + Na]^+$ (calcd for $C_{27}H_{30}O_{11}Na$, 553.1686), which was in agreement with the 1D NMR data (Table 1). The UV, IR, and NMR data of **2** were closely related to those of toonaciliatin H (**1**). The only difference was the C-12 of **2** being assigned as a ketone carbonyl at δ_C 210.2 instead of the oxymethine (δ_H 4.46, d, J = 4.1 Hz; δ_C 71.8) in **1**. This was confirmed by the HMBC correlations from H-9, H-11, H-17, and Me-18 to C-12. The relative configuration of **2** was established as identical to **1** on the basis of a ROESY experiment (see Supporting Information). Therefore, the structure of toonaciliatin I was established as **2**.

Toonaciliatin J (**3**) gave a molecular formula of $C_{25}H_{30}O_{10}$ as established on the basis of HRESIMS, indicating an *m*/*z* ion at 513.1727 $[M + Na]^+$ (calcd for $C_{25}H_{30}O_{10}Na$, 513.1737). The ^{1}H and ^{13}C NMR data of **3** were compatible to those of **1**, except that the proton and carbon resonances of the acetyl group were absent, and the H-6 at δ_H 4.71 was severely upfield-shifted compared to that of **1** (at δ_H 6.10), suggesting that a hydroxy group was located at C-6 in compound **3**. This conclusion was further verified by the HMBC correlations from H-6 to C-4, C-5, and C-7. Comprehensive analysis of 2D NMR (HSQC, HMBC, and ROESY) spectra further confirmed the structure of **3** (see Supporting Information).

Toonaciliatin K (**4**) was obtained as a white, amorphous powder. The HRESIMS displayed a pseudomolecular ion at *m*/*z* 539.3719 $[M + Na]^+$ (calcd for $C_{32}H_{52}O_5Na$, 539.3712) consistent with a molecular formula of $C_{32}H_{52}O_5$. IR absorption bands revealed the presence of hydroxy groups (3512, 3471 cm^{-1}) and a carbonyl group (1695 cm^{-1}). The ^{1}H NMR data (Table 2) indicated the presence of seven tertiary methyls at δ_H 0.84, 1.02, 1.04, 1.05, 1.13, 1.27, and 1.27 (each 3H, s), an *O*-ethyl at δ_H 3.76 and 3.43 (each 1H, m) and 1.23 (3H, t, J = 7.2 Hz), and one olefinic proton at δ_H 5.33 (1H, dd, J = 6.3, 2.9 Hz). The ^{13}C NMR spectrum showed 32 carbon resonances, which were classified by DEPT and HSQC experiments as one ketone carbonyl (δ_C 216.8), one trisubstituted double bond (δ_C 145.6 and 118.1), eight methyls, nine sp^3 methylenes, seven sp^3 methines, and five sp^3 quaternary carbons.

A comparison of its NMR data with those of (21*R*,23*R*)-epoxy-21α-ethoxy-24*S*, 25-dihydroxyapotirucalla-7-en-3-one (**4a**),[15] which was previously isolated from this plant genus, and also isolated in this investigation, indicated that **4** was an analogue of **4a**, an apotirucallene-type triterpenoid. Analysis of the ^{13}C NMR data (Table 2) of **4** and **4a** also revealed that the two compounds possessed the same tetracyclic A−D rings, and the only difference was likely the configuration of C-21 since the major changes of chemical shifts scattered around C-21. This was confirmed by the 2D NMR data (see Supporting Information). In the HMBC spectrum, the correlations of H-21/C-23 and OEt/C-21 revealed that C-21 and C-23 were linked via an oxygen atom to form a tetrahydrofuran ring, and the *O*-ethyl group was located at C-21 to form an acetal functionality. The HMBC correlations of H_2-22/C-24, H-24/C-25, Me-26/C-24, Me-26/C-25, and Me-27/C-25 placed two hydroxy groups at C-24 at δ_C 76.7 and C-25 at δ_C 72.9, respectively. The aforementioned evidence confirmed that **4** and **4a** shared the same planar structures. The ROESY spectrum showed that the relative configuration of the tetracyclic core in **4** was identical to that of **4a** (see Supporting Information). The chemical shifts from C-20 to C-27 in the side chain of **4** were compatible with those of agladupol A,[16] suggesting that 21-OEt was *β*-oriented, which was verified by the ROESY correlations of Me-18/H-21, H-21/H-12α, and Me-18/H-20. Furthermore, the C-17 and C-22

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **4**−**6** and ^{13}C NMR Data of **4a**[a,b]

	4	**4a**		**5**		**6**	
no.	δ_H, mult (*J* in Hz)	δ_C	δ_C	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C
1α	1.48, m	38.5	38.5	1.44, m	38.5	0.87, m	39.7
1*β*	1.97, m			1.97, m		1.80 m	
2α	2.26, dt (14.2, 3.6)	34.9	34.9	2.23, m	34.9	1.44, td (13.4, 4.2)	18.9
2*β*	2.77, td (14.2, 5.5)			2.76, td (14.5, 5.5)		1.90, m	
3α		216.8	216.6		216.8	1.00, m	37.9
3*β*						2.16, m	
4		47.9	47.9		47.8		43.7
5	1.73, m	52.4	52.4	1.71, m	52.3	1.11, dd (12.5, 2.1)	57.0
6	2.09, m	24.4	24.4	2.08, m	24.3	α 1.77, m	19.2
						β 2.09, m	
7	5.33, dd (6.3, 2.9)	118.1	118.1	5.30, m	118.1	α 1.29, m	43.4
						β 1.68, m	
8		145.6	145.6		145.5		72.4
9	2.32, m	48.3	48.5	2.27, m	48.4	0.82, m	56.2
10		35.1	35.1		35.0		37.7
11α	1.60, m	17.9	17.8	1.57, m	18.2	1.53, m	17.3
11*β*				1.63, m		1.64, m	
12α	1.66, m	31.7	31.7	1.62, m	33.5	1.30, m	38.2
12*β*	1.88, m			1.78, m		1.57, m	
13		43.5	43.7		43.6		36.4
14		50.8	51.0		51.3	1.33, m	51.5
15	1.56, m	34.2	33.8	1.55, m	33.9	5.73 dd (17.4, 10.6)	151.6
16α	1.33, m	27.3	27.5	1.26, m	28.3	4.87 dd (17.4, 1.1)	108.6
16*β*	1.89, m			1.89, m		4.81 dd (10.6, 1.1)	
17	1.98, m	46.2	50.4	1.55, m	53.0	1.22, s	24.3
18	0.84, s	23.2	22.6	0.84, s	22.0	1.24, s	28.9
19	1.02, s	12.7	12.7	0.99, s	12.9		183.5
20	2.11, m	45.0	47.8	2.03, m	33.3	0.90, s	13.7
21	4.81, d (3.0)	103.6	107.8	0.88, d (5.9)	19.4		
22	1.96, m	31.1	34.4	2.44, dd (15.4, 2.9)	45.5		
				2.16, m			
23	4.41, m	78.6	76.7		209.9		
24	3.18, brs	76.7	75.5	4.25, d (18.8)	68.9		
				4.14, d (18.8)			
25		72.9	73.0				
26	1.27, s	26.3	26.4				
27	1.27, s	26.3	26.3				
28	1.05, s	24.5	24.5	1.03, s	24.5		
29	1.13, s	21.5	21.5	1.10, s	21.6		
30	1.04, s	27.4	27.3	1.00, s	27.4		
21-OEt	3.76, m; 3.43, m;	63.7	63.9				
	1.23, t (7.2)	15.2	15.4				

[a] ^{1}H and ^{13}C NMR data were recorded at 400 and 100 MHz, respectively. [b] All the data were measured in $CDCl_3$.

resonances of **4** were upfield-shifted ca. $\Delta\delta$ 4.2 and 3.3 ppm, respectively, as compared to those of **4a**, due largely to the γ-gauche effects of 21-OEt.[17] The structure of **4** was thus established as depicted.

Toonaciliatin L (**5**) had a molecular formula of $C_{27}H_{42}O_3$, as deduced from the HREIMS at *m/z* 414.3146 $[M]^+$ (calcd 414.3134), and was corroborated by the positive-mode ESIMS ion at *m/z* 437.2 $[M + Na]^+$ and 851.6 $[2M + Na]^+$. The IR absorption bands implied the presence of hydroxy (3475 cm^{-1}) and carbonyl (1707 cm^{-1}) functionalities. The ^{1}H NMR spectrum of **5** showed the presence of five tertiary methyls at δ_H 0.84, 0.99, 1.00, 1.03, and 1.10 (each 3H, s), a secondary methyl at δ_H 0.88 (3H, d, $J = 5.9$ Hz), an olefinic proton at δ_H 5.30 (1H, m), and an oxymethene (δ_H 4.25, 1H, $J = 18.8$ Hz; δ_H 4.14, 1H, $J = 18.8$ Hz). The ^{13}C NMR spectrum displayed 27 carbon resonances, which were classified by DEPT and HSQC experiments as six methyls, nine sp^3 methylenes (one oxygenated), four sp^3 methines, four sp^3 quaternary carbons, one trisubstituted double bond, and two ketone carbonyls (δ_C 209.9 and 216.8). Comparison of the NMR data of **5** with those of dyvariabilin H[9] revealed that the structures of the two compounds were closely related, with the only difference being the C-17 side chain. In the HMBC spectrum, the C-21 methyl group was assigned by the HMBC correlations of Me-21/C-17, C-20, and C-22, which also indicated the linkages between C-17 and C-20 and between C-20 and C22; the HMBC correlations from H_2-22 to C-23 (δ_C 209.9) and C-24 (δ_C 68.9) permitted connection of C-22 and C-24 via the C-23 carbonyl, which was verified by the HMBC correlations of H-24/C-23 and OH-24/C-24 and C-23. Compound **5** shared the same relative configuration as that of tirucallane-type compounds. The ROESY correlations of Me-19/H-11*β*, Me-19/H-1*β*, Me-29/H-2*β*, Me-30/H-12*β*, Me-30/H-16*β*, Me-30/H-17, and Me-21/H-17 indicated that they were cofacial and were randomly assigned *β*-orientations. Subsequently, the ROESY correlations of Me-18/H-9, Me-18/H-16α, Me-18/H-20, Me-28/H-5, H-5/H-9, and H-5/H-1α suggested that they were α-oriented.

Toonaciliatin M (**6**) gave a molecular formula of $C_{20}H_{32}O_3$, as established on the basis of the HREIMS at *m/z* 320.2348 $[M]^+$ (calcd 320.2351). The IR absorption bands implied the presence of hydroxy (3403 cm^{-1}) and carbonyl (1690 cm^{-1}) functionalities. The ^{1}H NMR spectrum showed resonances for three tertiary methyls at δ_H 0.90, 1.22, and 1.24 (each 3H, s) and a terminal double bond at δ_H 4.81 (1H, dd, $J = 10.6$, 1.1 Hz), 4.87 (1H, dd, $J = 17.4$, 1.1 Hz), and 5.73 (1H, dd, $J = 17.4$, 10.6 Hz). The ^{13}C NMR spectrum with DEPT experiments resolved 20 carbon resonances comprising three methyls, eight sp^3 methylenes, two sp^3 methines, four sp^3 quaternary carbons (one oxygenated), one carboxyl, and a terminal double bond. The aforementioned data implied that compound **6** was a pimaradiene-type diterpenoid.[18] Comparison of the ^{13}C NMR data of **6** with those of 8*β*-hydroxypimar-15-en-19-oic acid[18b] revealed that the main differences occurred at C-15, C-16, and C-17. The HMBC correlations (see Supporting Information) from Me-17 to C-12, C-13, C-15, and C-16 indicated that the two compounds

had the same planar structures, suggesting **6** was a C-13 epimer of 8β-hydroxypimar-15-en-19-oic acid. Compared to the corresponding chemical shifts of 8β-hydroxypimar-15-en-19-oic acid, an upfield shift was observed for C-17 ($\Delta\delta$ 7.9 ppm) and downfield shifts for C-15 ($\Delta\delta$ 4.3 ppm) and C-16 ($\Delta\delta$ 5.9 ppm), indicating that the vinylic group was in the equatorial position and the Me-17 in an axial position.[19] This conclusion was confirmed by ROESY correlations (see Supporting Information) of Me-17/11β and Me-17/12β. The structure of **6** was therefore established.

The known compounds 5α,6β,8α-trihydroxy-28-norisotoonafolin,[5b] toonaciliatin C,[5b] bourjotinolone B,[8] dyvariabilin H,[9] meliatetraolenone,[10] (21*R*,23*R*)-epoxy-21α-ethoxy-24*S*,25-dihydroxyapotirucalla-7-en-3-one (**4a**),[11] 21α-ethoxy-24*S*,25-dihydroxyapotirucalla-7-en-3-one,[12] bourjotinoline A,[13] hispidone,[14] and 3-episapelin A[14] were identified by comparison of their spectroscopic data with reported data.

All the compounds were tested for antimicrobial activities against 11 microbes *in vitro*. Toonaciliatin M (**6**) showed moderate antifungal activity against *Trichophyton rubrum* with an MIC of 12.5 μg/mL, and amphotericin B was used as the positive control (MIC = 1.56 μg/mL). Bourjotinoline A exhibited antibacterial activity against *Staphylococcus epidermidis* ATCC 12228 with an MIC value of 6.25 μg/mL, where ofloxacin was the positive control (MIC = 0.39 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. 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 acquired on a Bruker AM-400 spectrometer. EIMS and HREIMS (70 eV) were carried out on a Finnigan MAT 95 mass spectrometer. ESIMS and HRESIMS were obtained on an Esquire 3000plus (Bruker Daltonics) and a Finnigan LC QDECA instrument, respectively. Silica gel (200−300 mesh) and silica gel H (Qingdao Haiyang Chemical Co. Ltd.), C18 reverse-phased silica gel (150−200 mesh, Merck), MCI gel (CHP20P, 75−150 μM, Mitsubishi Chemical Industries Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. Precoated silica gel GF254 plates (Qingdao Marine Chemical Plant, Qingdao, China) were used for TLC. All solvents used for chromatography were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China).

Plant Material. The leaves and twigs of *T. ciliata* Roem. were collected from Hainan Province, China, in October 2005 and were authenticated by Prof. Shi-Man Huang of the Department of Biology, Hannan University of China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (accession number: AMTA-tzzf-02Y).

Extraction and Isolation. The air-dried powder of leaves and twigs of *T. ciliata* (6 kg) was extracted three times with 95% EtOH at room temperture to give an ethanolic extract (500 g), which was partitioned between EtOAc and H_2O to obtain the EtOAc-soluble fraction (240 g). The EtOAc-soluble fraction was chromatographed on a silica gel column eluted with a mixture of petroleum ether/acetone (25:1 to 100% acetone, v/v) as a gradient to give three major fractions, 1−3. Fraction 1 (30 g) was separated on a column of MCI gel (MeOH/H_2O, 40:60 to 90:10, v/v) to afford fractions 1a−1c. Fraction 1a (5.3 g) was subjected to a silica gel column eluted with $CHCl_3$/MeOH (30:1, v/v) to give two major subfractions, each of which was purified on a column of reversed-phase silica gel eluted with MeOH/H_2O (60:40, v/v) to yield compound **3** (12 mg) and 5α,6β,8α-trihydroxy-28-norisotoonafolin (4 mg), respectively. Fraction 1b (3.5 g) was chromatographed on a silica gel column (petroleum ether/EtOAc, 2:1, v/v) to afford three major fractions, 1b1 (240 mg), 1b2 (420 mg), and 1b3 (300 mg). Fractions 1b1−1b3 were respectively purified by a column of reversed-phase silica gel eluted with MeOH/H_2O (60:40, v/v) to give compounds **1** (9 mg) and **2** (4 mg) and toonaciliatin C (6 mg). Fraction 2 (35 g) was fractionated on a column of MCI gel eluted with MeOH/H_2O (40:60 to 90:10, v/v) to afford three fractions, 2a−2c. Fraction 2a (3.6 g) was separated on a silica gel column eluted with petroleum ether/EtOAc (10:1, v/v) to give three major subfractions, and each of them was then purified on a column of reversed-phase C18 silica gel eluted with MeOH/H_2O (75:35, v/v) to yield **6** (4 mg), (21*R*,23*R*)-epoxy-21α-ethoxy-24*S*,25-dihydroxyapotirucalla-7-en-3-one (**4a**) (100 mg), and meliatetraolenone (80 mg), respectively. Fraction 2b (330 mg) was subjected to a silica gel column eluted with petroleum ether/EtOAc (2:1, v/v) to give two subfractions, 2b1 and 2b2. Fraction 2b1 was separated on a column of reversed-phase C18 silica gel (MeOH/H_2O, 75:35, v/v) to give compounds **4** (5 mg) and **5** (6 mg) and 21α-ethoxy-24*S*,25-dihydroxyapotirucalla-7-en-3-one (8 mg). Fraction 2b2 was processed with the same procedure as 2b1 to obtain bourjotinolone B (8 mg) and dyvariabilin H (40 mg). Fraction 2c was chromatographed on a column of silica gel (petroleum ether/EtOAc, 20:1, v/v) to afford three major fractions, 1c1 (240 mg), 1c2 (430 mg), and 1c3 (300 mg). Each of fractions 1c1−1c3 was purified on a column of reversed-phase C18 silica gel eluted with MeOH/H_2O (8:2, v/v) to afford bourjotinoline A (51 mg), 3-episapelin A (25 mg), and hispidone (5 mg), respectively.

Antimicrobial Tests. The *in vitro* antibacterial tests against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Escherichia coli* ATCC 25922, *Shigella flexneri*, *Pseudomonas aeruginosa* ATCC 2785, and *Bacillus subtilis* CMCC 63501 were conducted as described previously.[20] The microbial cells were suspended in Mueller-Hinton broth to form a final density of 5×10^{-5}−10^{-6} cfu/mL and incubated at 37 °C for 18 h under aerobic conditions with the respective compounds, which were dissolved in DMSO. The blank controls of microbial culture were incubated with limited DMSO under the same conditions. DMSO was determined not to be toxic at levels used in the experiments.

The *in vitro* antifungal activities against *Candida albicans* ATCC 1600, *Saccharomyces sake*, *Trichophyton rubrum*, and *Microsporum gypseum* were completed as described previously.[21] The fungi were incubated in Sabouraud dextrose broth at 37 °C for 48 h with the respective compounds, and the positive control was dissolved in DMSO. The blank controls of fungal cultures were incubated with limited DMSO under the same conditions.

Toonaciliatin H (1): white powder; $[\alpha]^{21}_D$ +156.0 (*c* 0.150, MeOH); UV (MeOH) λ_{max} (log ε) 211.8 (3.79) nm; IR (KBr) ν_{max} 3442, 2929, 1776, 1383, 1221, 1034, 957 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; positive mode ESIMS *m/z* 555.2 [M + Na]$^+$, 1087.4 [2 M + Na]$^+$; negative mode ESI MS *m/z* 531.4 [M − H]$^-$, 1063.4 [2 M − H]$^-$; EIMS *m/z* 514 (2), 496 (5), 478 (6), 454 (10), 279 (16), 243 (25), 205 (32), 190 (66), 175 (100), 146 (61), 137 (40), 109 (26), 95 (34), 77 (26), 55 (21); HRESIMS *m/z* 555.1824 (calcd for $C_{27}H_{32}O_{11}Na$, 555.1842).

Toonaciliatin I (2): white powder; $[\alpha]^{21}_D$ +172.0 (*c* 0.130, MeOH); UV (MeOH) λ_{max} (log ε) 212.0 (3.72) nm; IR (KBr) ν_{max} 3471, 3348, 1794, 1749, 1718, 1379, 1219, 1032, 957 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; positive mode ESIMS *m/z* 553.2 [M + Na]$^+$, 1083.3 [2 M + Na]$^+$; negative mode ESIMS *m/z* 529.8 [M − H]$^-$, 1059.6 [2 M − H]$^-$; EIMS *m/z* 512 (1), 494 (1), 470 (3), 452 (5), 386 (7), 321 (9), 279 (11), 231 (12), 205 (29), 189 (100), 173 (58), 147 (51), 121 (20), 109 (20), 91 (35), 77 (26), 55 (17); HRESIMS *m/z* 553.1666 (calcd for $C_{27}H_{30}O_{11}Na$, 553.1686).

Toonaciliatin J (3): white powder; $[\alpha]^{21}_D$ +107.0 (*c* 0.245, MeOH); UV (MeOH) λ_{max} (log ε) 213.0 (3.23) nm; IR (KBr) ν_{max} 3385, 1767, 1637, 1080, 1018, 791, 600 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; positive mode ESIMS *m/z* 1003.3 [2 M + Na]$^+$; negative mode ESIMS *m/z* 489.3 [M − H]$^-$, 979.6 [2 M − H]$^-$; EIMS *m/z* 473 (1), 454 (3), 398 (10), 380 (8), 360 (7), 279 (10), 167 (28), 149 (100), 84 (37), 71 (28), 57 (39); HRESIMS *m/z* 513.1727 (calcd for $C_{25}H_{30}O_{10}Na$, 513.1737).

Toonaciliatin K (4): white powder; $[\alpha]^{21}_D$ +10.0 (*c* 0.050, $CHCl_3$); IR (KBr) ν_{max} 3512, 3471, 2968, 1695, 1377, 1105, 1036, 974, 906 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; positive mode ESIMS *m/z* 539.5 [M + Na]$^+$ and 1055.8 [2 M + Na]$^+$; EIMS *m/z* 499 (1), 470 (12), 455 (19), 440 (49), 427 (100), 397 (45), 365 (59), 337 (20), 313 (31), 297 (66), 271 (28), 245 (25), 159 (38), 145 (40), 133 (44), 105 (51), 95 (57), 69 (34), 59 (52); HRESIMS *m/z* 539.3719 [M + Na]$^+$ (calcd for $C_{32}H_{52}O_5Na$, 539.3712).

Toonaciliatin L (5): white powder; $[\alpha]^{21}_D$ −77.0 (*c* 0.065, MeOH); IR (KBr) ν_{max} 3475, 2964, 1707, 1441, 1387, 1105, 1057. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; EIMS *m/z* 414 (27), 399 (100), 381 (16), 325 (77), 187 (11), 173 (13), 159 (16), 147 (13), 121 (27), 95 (43), 81 (33); HRESIMS *m/z* 414.3146 (calcd 414.3134).

Toonaciliatin M (6): white powder; $[\alpha]^{21}_{D}$ +20.0 (*c* 0.125, $CHCl_3$); IR (KBr) ν_{max} 3403, 2925, 1690, 1637, 1477, 1271, 1181, 908; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; EIMS *m*/*z* 320 (10), 306 (20), 305 (100), 302 (65), 287 (16), 274 (23), 241 (19), 209 (33), 148 (32), 121 (40), 107 (27), 95 (26), 79 (25); HRESIMS *m*/*z* 320.2348 (calcd 320.2351).

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Supporting Information Available: Key HMBC correlations of **1**−**5** (figures), selected ROESY correlations of **1** and **4**−**6** (figures); IR, EIMS, ^1H, ^{13}C NMR, and 2D NMR spectra of toonaciliatin H−M (**1**−**6**). This material is available free of charge via the Internet at http://pubs.acs.org.

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