# Bioactive Limonoid and Triterpenoid Constituents of *Turraea pubescens*

Chun-Mao Yuan,[†,‡] Gui-Hua Tang,[†] Yu Zhang,[†] Xiao-Ying Wang,[†] Ming-Ming Cao,[†] Feng Guo,[†] Yan Li,[†] Ying-Tong Di,[†] Shun-Lin Li,[†] Hui-Ming Hua,*[,‡] Hong-Ping He,*[,†] and Xiao-Jiang Hao*[,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

[‡]Key Laboratory of Structure-Based Drug Design and Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China

Ⓢ *Supporting Information*

ABSTRACT: Eleven new limonoids, turrapubins A–K (**1–11**), and three new triterpenoids (**12–14**), along with 14 known compounds, were isolated from the twigs of *Turraea pubescens*. The structures of **1–14** were elucidated on the basis of NMR and MS analysis. Compounds **12**, **16**, **18**, and **19** exhibited inhibitory activities against lipopolysaccharide-induced nitric oxide production in RAW264.7 cells. In addition, compounds **2**, **11**, **18**, and **26** exhibited inhibitory activities against brine shrimp larvae (*Artemia salina*) at 100 ppm with the corrected mortality ranging from 81.7% to 100%.

RESULTS AND DISCUSSION
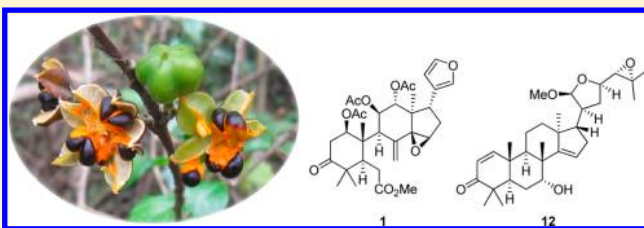
Limonoids are a class of highly oxygenated nortriterpenoids and exhibit a wide range of bioactivities such as insect antifeedant, cytotoxic, antifungal, anti-inflammatory, and antimalarial effects.[1] *Turraea pubescens* Hellen (Meliaceae), a shrub occurring in sparse forests near the ocean, is native to mainland China.[2] In Chinese folkloric medicine, the twigs and leaves of *T. pubescens* have been used for the treatment of dysentery, pharyngolaryngitis, and traumatic hemorrhage.[3] Previous phytochemical investigations on plants in the genus *Turraea* have led to the identification of a series of limonoid, triterpenoid, and pregnane derivatives.[4] Recently, our group has reported an array of limonoids with varied structural features and different types of biological activities from species in the Meliaceae family.[5] In the present study, 11 new limonoids, turrapubins A–K (**1–11**), and three new triterpenoids (**12–14**), as well as 14 known compounds, were isolated from the twigs of *T. pubescens*. In this paper, the isolation and structure elucidation of the new compounds as well as the biological evaluation of these compounds are reported.

RESULTS AND DISCUSSION

Turrapubin A (**1**) was obtained as a white, amorphous powder, and its molecular formula, $C_{33}H_{42}O_{11}$, was deduced from the molecular ion peak $[M]^+$ at *m/z* 614.2733 (calcd for $C_{33}H_{42}O_{11}$, 614.2727) in the HREIMS. Its IR spectrum suggested the presence of hydroxy (3441 cm^{-1}) and carbonyl (1735 cm^{-1}) groups. The ^{13}C NMR data along with DEPT experiments showed 33 carbon signals, including eight methyls, four methylenes, 10 methines (three olefinic), and 11 quaternary carbons (two olefinic and five carbonyl). Comparison of the NMR spectroscopic data (Table 1) with those of 11-*epi*-toonacilin[6] indicated that **1** is a ring B-*seco* limonoid, bearing

the same C, D, E, and 13,14-epoxy rings. The chemical shift differences resulted from the absence of Δ^1 double-bond signals and the presence of an *O*-bearing CH resonance (δ_H 5.56, d, *J* = 3.8 Hz; δ_C 78.0). However, it was not possible to assign the location of two acetyl groups, due to the absence of any HMBC correlations between the Me-19 signal (δ_H 1.65, s) with the other carbon resonances and between the two oxygenated methine resonances (δ_H 5.56, d, *J* = 3.8 Hz and 5.70, d, *J* = 11.5 Hz) with the corresponding acetyl groups, when measured at room temperature. Therefore, the NMR spectra were performed again at a lower temperature of 276 K. As a result, the HMBC correlations from Me-19 and H-9 to C-1 (δ_C 78.0) and from H-1 and H-12 to their corresponding acetyl carbonyls, along with the analysis of the $^1H–^1H$ COSY cross-peak between H-1/H$_2$-2, suggested that two acetyl groups are located at C-1 and C-12, respectively (Figure 1). The ketone carbonyl was assigned at C-3, which was confirmed by the HMBC correlations from H-1, Me-28, and Me-29 to C-3 (δ_C 212.0). Thus, the gross structure of **1** was constructed as depicted.

The relative configuration of **1** was determined from the ROESY spectrum, in which correlations of Me-19 with Me-29 and H-12 and between H-12 and H-17 indicated that those groups are cofacial, and they were assigned arbitrarily as *β*-oriented (Figure 1). In addition, the ROESY cross-peaks of H-9/H-1, H-9/H-11, H-9/H-5, H-5/Me-28, and H-11/Me-18 suggested the *α*-orientation of those groups. These results coincided well with the large coupling constants between key
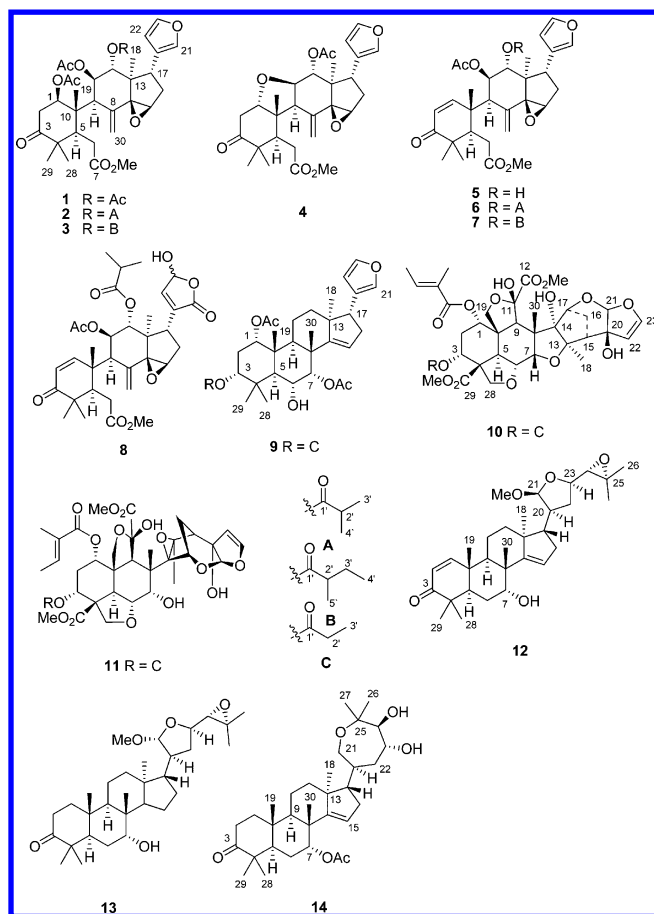
protons ($J_{9,11}$ = 7.0 Hz and $J_{11,12}$ = 11.5 Hz). Therefore, the relative configuration of **1** was established as shown.

Turrapubin B (**2**) was found to possess the molecular formula $C_{35}H_{46}O_{11}$, by analysis of its HREIMS. The ^1H NMR and ^{13}C NMR spectra (Table 1) were very similar to those of **1**. The most noticeable differences were the presence of an isobutanoyloxy moiety and the absence of an acetyl group in **2**. The isobutanoyloxy group signals (δ_H 0.80, d, J = 7.0 Hz, 0.84, d, J = 7.0 Hz, and 2.07, m; δ_C 18.4, 18.5, 34.1, and 176.1) were apparent from the ^1H NMR and ^{13}C NMR data, which were confirmed by the HMBC correlations from Me-3′, Me-4′, and H-2′ to C-1′. The isobutanoyloxy group was located at C-12 on the basis of the key HMBC cross-peaks of Me-18/C-12 and H-12/C-1′. Thus, compound **2** was determined as 12-deacetyl-12-isobutanoyloxylturrapubin A (turrapubin B).

The molecular formula of turrapubin C (**3**) was determined as $C_{36}H_{48}O_{11}$ from the HREIMS. Comparison of the ^1H and ^{13}C NMR spectroscopic data (Table 1) with those of **1** indicated that they possess a similar structure except for the presence of a 2-methylbutanoxy moiety in **3** instead of an acetyl group in the latter. In the HMBC spectrum, correlations of Me-4′/C-2′, Me′-5/C-3′, Me′-5/C-1′, and H-12/C-1′ implied that the 2-methylbutanoxy group was placed at C-12. The relative configurations of **2** and **3** were almost the same as that of **1** from the ROESY correlations. Accordingly, the structure of **3** (turrapubin C) was determined as 12-deacetyl-12-(2-methylbutanoxyl)turrapubin A.

Compounds **1**−**3** were investigated by means of their 2D-NMR data observed at a low temperature of 276 K. The key HMBC correlations between the Me-19 proton signal and the other carbon resonances and between two oxygenated methine resonances and the corresponding acetyl groups in compounds **1**−**3** could not be observed when measured at room temperature. This may result from the rotation of C-9/C-10 at room temperature.

Turrapubin D (**4**) gave the molecular formula $C_{29}H_{36}O_8$ from the HREIMS, requiring 12 indices of hydrogen deficiency of unsaturation. The ^1H and ^{13}C NMR spectra of **4** (Tables 2 and 3) exhibited resonances for four tertiary methyls, an α-substituted furan, an exocyclic $\Delta^{8(30)}$ double bond, a carbomethoxy, and an acetyl group. The NMR spectroscopic data were analogous to those of **1**. The major differences were the replacement of two acetyl group in **1** with a five-membered ring through an oxygen atom between C-1 and C-11 in **4**. The HMBC correlations of Me-19/C-1 and H-1/C-11, along with the ^1H−^1H COSY cross-peaks of H-1/H$_2$-2 and H-11/H-12, enabled the establishment of the 1,11-epoxy ring (Supporting Information).

The relative configuration of **4** was assigned by the ROESY spectrum, in which correlations of Me-19/H-1, H-1/H-12, and H-12/H-17 indicated these groups to be β-oriented. In addition, H-5, H-9, H-11, Me-18, and Me-28 were assigned as α-oriented by the ROESY correlations from H-5 to H-9 and Me-28 and from H-11 to Me-18 (Supporting Information). Thus, the relative configuration of **4** was established as shown.

The molecular formula of turrapubin E (**5**) was assigned as $C_{29}H_{36}O_8$ from the HREIMS, 42 mass units less than 11-*epi*-toonacilin.[6] The UV absorption band at 235 nm was suggestive of an α,β-unsaturated ketone group.[7] A detailed analysis of the NMR data suggested that **5** shares the same skeleton as 11-*epi*-toonacilin.[6] Comparison of the chemical shift of H-12 at δ_H 4.28 (dd, J = 10.3, 6.0 Hz) of **5** with that of 11-*epi*-toonacilin at δ_H 5.68 (d, J = 10.8 Hz) implied that an α-acetyl group at C-12 in the latter is replaced by an α-hydroxy group in **5**. This assignment was verified by the HMBC correlation of Me-18/H-12 along with the ^1H−^1H COSY correlation of H-11/H-12 (Supporting Information). In the ROESY spectrum, a correlation between H-17 and H-12 as well as the large coupling constant (J = 10.3 Hz) between H-11 and H-12 was used to assign an α-oriented hydroxy group at C-12 (Supporting Information). Thus, the structure of **5** was established as shown.

The HREIMS and ^{13}C NMR data of **6** revealed a molecular formula of $C_{33}H_{42}O_9$. The 1D-NMR data of **6** displayed a similar pattern to **5** except for the presence of an isobutanoyloxy group replacing a hydroxy group. The isobutanoyloxy group was linked to C-12, which was deduced from the key HMBC correlations of Me-3′ (δ_H 0.86, d, J = 5.6 Hz), Me-4′ (δ_H 0.85, d, J = 5.6 Hz), and H-2′ (δ_H 2.10, m) with C-1′ (δ_C 175.6) and of H-12 (δ_H 5.71, d, J = 10.8 Hz) with C-1′. Accordingly, the structure of **6** was elucidated as 12-deacetoxy-12-isobutanoyloxytoonacilin (turrapubin F).

Turrapubin G (**7**) was found to possess a molecular formula of $C_{34}H_{44}O_9$, by means of HREIMS, 14 mass units more than **6**. The ^1H and ^{13}C NMR spectroscopic data (Tables 2 and 3) indicated that it is structurally similar to **6**, with the only difference being the presence of a 2-methylbutanoxy moiety at C-12, rather than an isobutanoyloxy group. This conclusion was further confirmed by the HMBC correlations of Me-4′/C-2′, Me′-5/C-3′, Me′-5/C-1′, and H-12/C-1′. Therefore, the structure of **7** was elucidated as 12-deacetoxy-12-(2-methylbutanoxyl)toonacilin (turrapubin G).

The molecular formula of turrapubin H (**8**), $C_{33}H_{42}O_{11}$, was determined from the HREIMS. An analysis of the 1D-NMR

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds 1−3 at 276 K (600 and 150 MHz, in CDCl$_3$)

|	1		2		3	
position	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type
1	5.56, d (3.8)	78.0, d	5.51, d (4.0)	78.2, d	5.49, d (4.0)	78.3, d
2α	2.45, dd (14.7, 3.8)	37.2, t	2.48, dd (15.0, 4.0)	37.3, t	2.49, dd (14.8, 4.0)	37.2, t
2β	2.97, m		2.95, d (15.0)		2.96, m	
3		212.0, s		212.2, s		212.2, s
4		49.3, s		49.5, s		49.6, s
5	2.41, m	44.6, d	2.42, d (7.5)	44.8, d	2.42, d (7.5)	44.8, d
6a	2.24, m	31.5, t	2.23, m	31.7, t	2.22, m	31.7, t
6b	3.29, d (18.8)		3.33, d (18.9)		3.33, m	
7		174.7, s		175.0, s		175.0, s
8		134.4, s		134.6, s		134.7, s
9	3.39, d (7.0)	50.7, d	3.41, d (7.6)	50.8, d	3.41, d (7.8)	50.8, d
10		46.4, s		46.6, s		46.6, s
11	5.34, dd (7.0, 11.5)	72.0, d	5.38, dd (11.0, 7.6)	72.1, d	5.38, dd (11.0, 7.8)	72.2, d
12	5.70, d (11.5)	74.6, d	5.70, d (11.0)	74.5, d	5.68, d (11.0)	74.4, d
13		45.0, s		45.6, s		45.7, s
14		71.1, s		71.2, s		71.3, s
15	3.77, s	59.5, d	3.75, s	59.6, d	3.75, s	59.7, d
16α	1.81, dd (11.6, 13.8)	33.1, t	1.79, m	33.9, d	1.79, t (12.0)	34.1, t
16β	2.22, dd (13.8, 6.4)		2.18, m		2.19, m	
17	2.96, m	37.8, d	2.98, dd (10.5, 7.3)	37.8, d	2.99, m	37.8, d
18	0.84, s	13.1, q	0.90, s	13.5, q	0.90, s	13.5, q
19	1.65, s	17.2, q	1.64, s	17.3, q	1.64, s	17.4, q
20		122.1, s		122.4, s		122.4, s
21	7.06, s	140.0, d	7.05, s	140.4, d	7.05, s	140.5, d
22	6.09, s	111.1, d	6.13, s	111.3, d	6.13, s	111.4, d
23	7.27, s	142.5, d	7.25, s	142.7, d	7.25, s	142.6, d
28	0.93, d	25.0, q	0.93, s	25.2, q	0.93, s	25.2, q
29	1.10, s	24.2, q	1.10, s	24.4, q	1.10, s	24.4, q
30a	5.07, s	123.4, t	5.07, s	123.5, t	5.07, s	123.5, t
30b	5.36, s		5.36, s		5.36, s	
OMe-7	3.56, s	51.6, q	3.56, s	51.8, t	3.56, s	51.8, q
OAc-1		169.9, s		170.2, s		170.2, s
	1.94, s	21.0, q	1.95, s	21.2, q	1.95, s	21.2, q
OAc-11		170.3, s		175.0, s		170.6, s
	1.99, s	21.3, q	1.98, s	21.8, q	1.98, s	21.8, q
1′		170.4, s		176.1, s		175.7, s
2′	1.73, s	20.7, q	2.07, m	34.1, d	1.86, m	40.9, d
3′			0.80, d (7.0)	18.4, q	1.05, m	25.8, t
					1.35, m	
4′			0.84, d (7.0)	18.5, q	0.74, t (7.5)	12.0, q
5′					0.77, d (7.3)	15.3, q
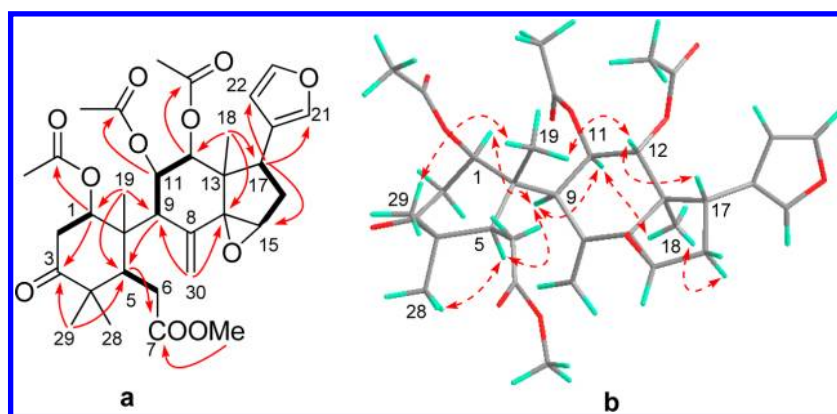
Figure 1. ^{1}H−^{1}H COSY (bold), key HMBC (a), and key ROESY (b) correlations of 1.

Table 2. ^{1}H NMR Spectroscopic Data for Compounds 4−9 in CDCl$_3$

position	4[a] δ_H (J in Hz)	5[a] δ_H (J in Hz)	6[a] δ_H (J in Hz)	7[a] δ_H (J in Hz)	8[a] δ_H (J in Hz)	9[b] δ_H (J in Hz)
1	4.12, t (5.7)	7.38, d (10.6)	7.46, d (10.6)	7.42, d (10.6)	7.42, d (10.3)	4.53, t (3.4)
2α	2.85, dd (14.9, 5.7)	6.06, d (10.6)	6.17, d (10.6)	6.12, d (10.6)	6.18, d (10.3)	1.91, dt (16.4, 3.4)
2β	2.70, dd (14.9, 5.7)					2.23, m
3						4.68, t (3.4)
5	2.63, dd (7.5, 3.6)	3.08, dd (7.0, 1.9)	2.93, dd (7.8, 2.7)	2.87, dd (8.0, 2.1)	2.88, d (7.0)	2.27, d (11.5)
6a	2.49, m	2.31, m	2.33, dd (16.6, 2.7)	2.29, dd (16.9, 2.1)	2.32, m	4.35, dd (11.5, 2.4)
6b		2.46, m	2.45, dd (16.6, 7.8)	2.40, dd (16.9, 8.0)	2.43, dd (16.8, 7.0)	
7						5.29, d (2.4)
9	3.38, d (8.7)	2.95, d (7.2)	2.98, d (7.5)	2.94, d (7.2)	2.95, d (7.3)	2.51, m
11α	4.35, t (8.7)	5.40, dd (10.3, 7.2)	5.57, dd (10.8, 7.5)	5.54, dd (10.8, 7.2)	5.53, dd (10.4, 7.3)	1.50, m
11β						1.24, m
12α						1.77, m
12β	5.47, d (8.7)	4.28, dd (10.3, 6.0)	5.71, d (10.8)	5.66, d (10.8)	5.68, d (10.4)	1.51, m
15	3.87, s	3.88, s	3.87, s	3.83, s	3.89, s	5.37, d (1.5)
16α	1.80, m	1.90, m	1.82, dd (14.2, 10.9)	1.80, dd (13.6, 10.4)	2.17, m	2.40, m
16β	2.24, dd (14.0, 7.4)	2.32, m	2.23, dd (14.2, 7.1)	2.18, dd (13.6, 7.3)	2.21, m	2.33, m
17	2.99, dd (10.8, 7.4)	3.01, dd (10.4, 7.1)	3.08, dd (10.9, 7.1)	3.04, dd (10.4, 7.3)	2.96, m	2.76, dd (10.9, 7.3)
18	0.80, s	0.74, s	0.96, s	0.93, s	0.98, s	0.76, s
19	1.06, s	0.96, s	0.98, s	0.95, s	0.95, s	1.02, s
21	7.12, s	7.22, s	7.11, brs	7.07, s		7.21, s
22	6.16, s	6.39, s	6.17, m	6.14, s	6.86, s	6.24, s
23	7.32, s	7.31, s	7.29, t (1.4)	7.24, s	6.06, brs	7.35, t (1.5)
28	0.96, s	1.02, s	0.99, s	0.96, s	0.96, s	1.09, s
29	1.11, s	1.10, s	1.10, s	1.05, s	1.08, s	1.09, s
30a	5.30, s	5.18, s	5.22, s	5.18, s	5.23, s	1.20, s
30b	5.31, s	5.29, s	5.32, s	5.28, s	5.32, s	
OMe-7	3.71, s	3.68, s	3.68, s	3.63, s	3.66, s	
OAc-1						1.98, s
OAc-7						2.05, s
OAc-11		2.03, s	1.90, s	1.85, s	1.88, s	
OAc-12	1.89, s	12-OH 1.85 d (6.0)				
2′			2.10, m	1.87, m	2.29, m	2.23, m
						2.32, m
3′			0.86, d (5.6)	1.34, m	1.03, d (6.0)	1.12, t (7.7)
				1.10, m		
4′			0.85, d (5.6)	0.72, m	0.97, d (6.0)	
5′				0.73, m		

^{a}Recorded at 500 MHz. ^{b}Recorded at 600 MHz.

data suggested that the furan ring in **6** is oxygenated to a 4-hydroxy-α,β-unsaturated lactone (δ_H 6.06, brs and 6.86, s; δ_C 96.1, 135.3, 147.6, and 171.0) in **8**, which was supported by the HMBC correlations from H-23 to C-20, H-22 to C-21, and H-17 to C-20, C-21, and C-22 (Supporting Information). The relative configurations of turrapubins F−H (**6**−**8**) were identical to that of **5** on the basis of their ROESY correlations. The absolute configuration of **8** was assigned by comparing the CD spectrum with that of turrapubesin D,[7] a limonoid with an established absolute configuration. On the basis of the above data analysis, the structure of turrapubin H (**8**) was assigned as depicted.

Turrapubin I (**9**) gave the molecular formula C$_{33}$H$_{46}$O$_8$, as deduced from the HREIMS. The NMR data (Tables 2 and 3) were similar to those of mesendanin B.[8] The only difference observed was that an acetyl group in the latter was replaced by a propanoyl group in **9**. In the HMBC spectrum, correlations from H-3 (δ_H 4.68, t, J = 3.4 Hz), Me-3′ (δ_H 1.12, t, J = 7.7 Hz), and H$_2$-2′ (δ_H 2.23, m and 2.32, m) to the carbonyl of the propanoyl group revealed that the propanoyl group is attached to C-3. The α-oriented propanoyl group located at C-3 was deduced from the small coupling constant (J = 3.4 Hz) between H-3 and H$_2$-2. Thus, compound **9** was assigned as 3-deacetyl-3-propanoylmesendanin B.

The HREIMS of turrapubin J (**10**) indicated a molecular formula of C$_{36}$H$_{46}$O$_{16}$. The ^{13}C NMR spectrum displayed 36 carbons signals, comprising seven methyls (two methoxyls), five methylenes, 12 methines (three olefinic), and 12 quaternary carbons (one olefinic and four carbonyl). The aforementioned information indicated that turrapubin J has the same skeleton as 1-tigloyl-3-acetylazadirachtinin.[9] Compound **10** showed additional signals of a propanoyl group, rather than an acetyl group in its NMR spectra. The propanoyl group was assigned at C-3 by the HMBC correlations from H-3, H-2′ (2.18, m, 2H), and Me-3′ (δ_H 0.99, t, J = 7.3 Hz, 3H) to C-1′ (Supporting Information). The relative stereochemistry of **10** was assigned from the proton coupling constant analysis and the ROESY spectrum (Supporting Information). The small coupling constant ($J_{2,3}$ = 3.0 Hz) between H-3 and H$_2$-2

Table 3. ^{13}C NMR Spectroscopic Data for Compounds 4−9 in CDCl$_3$

position	4[a] δ_C, type	5[a] δ_C, type	6[a] δ_C, type	7[a] δ_C, type	8[a] δ_C, type	9[b] δ_C, type
1	82.6, d	153.1, d	152.4, d	152.5, d	152.5, d	72.9, d
2	40.0, t	125.2, d	125.7, d	125.6, d	125.7, d	26.1, t
3	213.4, s	204.2, s	204.0, s	203.9, s	204.4, s	77.2, d
4	48.6, s	45.8, s	46.2, s	46.2, s	46.2, s	36.9, s
5	49.2, d	45.0, d	45.1, d	45.1, d	45.1, d	43.4, d
6	31.9, t	31.3, t	31.3, t	31.3, t	31.3, t	69.1, d
7	174.8, s	174.1, s	174.1, s	174.1, s	174.2, s	80.5, d
8	138.7, s	137.1, s	136.8, s	136.8, s	136.2, s	43.0, s
9	56.6, d	52.9, d	52.9, d	52.9, d	52.8, d	35.3, d
10	48.7, s	42.0, s	42.0, s	42.0, s	42.0, s	41.7, s
11	80.6, d	74.3, d	71.3, d	71.3, d	71.5/71.4, d	16.1, t
12	76.7, d	75.9, d	74.6, d	74.5, d	74.6, d	33.0, t
13	45.1, s	45.8, s	45.5, s	45.6, s	46.1, s	47.4, s
14	72.0, s	71.8, s	71.1, s	71.1, s	71.1/71.0, s	158.9, s
15	59.5, d	59.7, d	59.5, d	59.4, d	59.7/59.5, d	119.8, d
16	34.1, t	32.2, t	33.9, t	34.0, t	30.8, t	34.4, t
17	38.2, d	39.1, d	37.6, d	37.6, d	38.4/38.2, d	51.5, d
18	14.1, q	12.3, q	13.7, q	13.8, q	13.5, q	20.4, q
19	18.3, q	21.3, q	21.1, q	21.1, q	21.2, q	17.4, q
20	123.2, s	123.2, s	122.2, s	122.1, s	135.3/135.1, s	124.7, s
21	140.6, d	140.1, d	140.4, d	140.5, d	171.0/170.9, s	139.9, d
22	111.6, d	112.1, d	111.2, d	111.2, d	147.7/147.4, d	111.2, d
23	142.9, d	142.5, d	142.4, d	142.3, d	96.1, d	142.8, d
28	25.6, q	23.0, q	23.0, q	23.0, q	22.9, q	30.6, q
29	21.1, q	22.7, q	22.7, q	22.7, q	22.7, q	22.3, q
30	119.7, t	120.5, t	120.6, t	120.6, t	121.3, t	27.5, q
OMe-7	52.6, q	52.1, q	52.1, q	52.1, q	52.2, q	
OAc-1						170.3, s
						21.4, q
OAc-7						172.1, s
						21.5, q
OAc-11		170.9, s	169.7, s	169.7, s	169.9, s	
		20.9, q	20.8, q	20.8, q	20.9, q	
OAc-12	171.3, s					
	21.7, q					
1′			175.6, s	175.1, s	175.7/175.6, s	173.7, s
2′			33.9, d	40.9, d	33.9/33.8, d	28.2, t
3′			18.3, q	25.7, t	18.4/18.2, q	9.4, q
4′			18.2, q	11.6, q	18.9/18.8, q	
5′				15.1, q		

[a]Recorded at 125 MHz. [b]Recorded at 150 MHz.

suggested that H-3 is β-oriented. Thus, compound **10** was determined as 1-tigloyl-3-propanoylazadirachtinin.

Turrapubin K (**11**) gave the same molecular formula as **10** according to the HREIMS. Its NMR spectroscopic data (Table 4) resembled those of azadirachtin.[10] The major difference was the replacement of the acetyl signals at C-3 with the propanoyl signals [δ_H 1.04 (t, J = 7.5 Hz, 3H), 2.16 (m, 1H), 2.27 (m, 1H); δ_C 9.3, 28.4, and 175.0] in **11**. The key HMBC correlations of H-3, H-2′, and Me-3′ with C-1′ placed the propanoyl group at C-3. The small coupling constant ($J_{2,3}$ = 2.8 Hz) between H$_2$-2 and H-3 suggested that H-3 is β-oriented. Thus, compound **11** was established as 3-deacetyl-3-propanoyladirachtin-A (turrapubin K).

Compound **12** gave the molecular formula C$_{31}$H$_{46}$O$_5$ from the HREIMS. An α,β-unsaturated ketone group (δ_C 125.7, 158.5, and 205.5), a double bond (δ_C 120.1 and 161.7), a five-membered hemiacetal ring (δ_C 104.4 and 78.8), a trisubstituted

epoxide moiety (δ_H 2.71, d, J = 8.0 Hz; δ_C 57.5 and 67.8), and seven methyls were evident from its 1D-NMR spectroscopic data (Table 5). The above information indicated that **12** is an apotirucallane-type triterpenoid.[11] The ^{1}H and ^{13}C NMR data of **12** were closely related to those of bruceajavanin B.[11a] The presence of a hydroxy group at C-7 in **12** instead of an acetyl group in the latter was observed, which was supported by analysis of the upfield signal for C-7 (δ_C 71.7) as well as the HMBC correlations of Me-30 and H-5 with C-7 (Supporting Information). The ROESY correlation of Me-30/H-7 indicated that H-7 could be assigned as β-oriented, suggesting that OH-7 is α-oriented (Supporting Information). Therefore, compound **12** was determined as 7-deacetylbruceajavanin B.

The molecular formula of compound **13**, C$_{31}$H$_{48}$O$_5$, was deduced from the HREIMS, two mass units more than **12**. Inspection of the NMR data led to the conclusion that this compound is also an apotirucallane-type triterpenoid[11] with a

Table 4. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds 10 and 11 (500 and 125 MHz)

position	10[a] δ_H (J in Hz)	10[a] δ_C, type	11[b] δ_H (J in Hz)	11[b] δ_C, type
1	5.16, s	71.2, d	4.68, s	72.7, d
2α	2.57, dd (16.6, 3.0)	30.3, t	2.31, m, 2H	30.3, t
2β	2.43, dd (16.6, 3.0)			
3	5.75, t (3.0)	67.5, d	5.42, t (2.8)	68.6, d
4		53.4, s		53.9, s
5	3.62, m	37.5, d	3.62, m	38.1, d
6	4.84, dd (12.5, 3.0)	72.8, d	4.54, dd (12.3, 2.0)	75.8, d
7	4.97, d (3.0)	83.1, d	4.49, s	75.4, d
8		51.8, s		47.5, s
9	4.10, s	47.3, d	3.51, m	46.2, d
10		50.1, s		52.1, s
11		105.0, s		105.6, s
12		171.8, s		173.3, s
13		95.7, s		70.7, s
14		93.7, s		72.7, s
15	4.42, s	81.9, d	4.66, m	77.7, d
16a	1.99, m	30.2, t	1.20, m	26.8, t
16b	2.33, m		1.68, m	
17	2.29, m	51.3, d	2.30, m	49.4, d
18	1.88, s	27.6, q	1.94, s	19.5, q
19a	3.98, d (8.8)	69.7, t	3.62, d (9.0)	69.9, t
19b	4.49, d (8.8)		4.10, d (9.0)	
20		87.0, s		83.8, s
21	6.08, s	110.1, d	5.64, s	108.9, d
22	5.09, d (2.8)	109.2, d	5.08, d (2.6)	109.5, d
23	6.59, d (2.8)	146.0, d	6.46, d (2.6)	147.5, d
28a	4.02, d (8.4)	73.1, t	3.79, d (8.9)	73.8, t
28b	4.25, d (8.4)		3.96, d (8.9)	
29		173.6, s		175.2, s
30	2.02, s	17.6, q	1.63, s	22.4, q
OMe-12	3.62, s	52.8, q	3.59, s	53.4, q
OMe-29	3.65, s	52.7, q	3.80, s	53.1, q
tigloyl-1		167.0, s		168.1, s
		129.1, s		129.6, s
	7.24, m	138.3, d	7.13, m	140.1, d
	1.85, d (7.3)	14.2, q	1.81, d (6.7)	14.5, q
	1.98, s	12.4, q	1.86, s	12.1, q
propanoyl-3		173.8, s		175.0, s
	2.18, m	27.7, t	2.27, m	28.4, t
			2.16, m	
	0.99, t (7.3)	9.2, q	1.04, t (7.5)	9.3, q

[a]Recorded in pyridine-d_5. [b]Recorded in methanol-d_4.

similar structure to that of **12**. The HMBC cross-peaks of Me-19 and H-5 with C-1 (δ_C 38.5) as well as the ^{1}H–^{1}H COSY correlation between H$_2$-1 (δ_H 1.52, m and 1.87, m) and H$_2$-2 (δ_H 2.44, m and 2.53, m) confirmed the absence of the $\Delta^{1(2)}$ double bond. In addition, the ROESY correlation of H-21/H-17 along with the downfield-shifted carbon signal of C-21 (from δ_C 104.1 to δ_C 109.1) implied that the OMe-21 of **13** is α-oriented, in agreement with the literature.[11b] The ROESY correlations were almost the same as those of **12**. Therefore, compound **13** was established as 7-deacetyl-21α-methoxy-dihydrobruceajavanin B.

Compound **14** was shown to possess the molecular formula $C_{32}H_{50}O_6$, by means of HREIMS. Analysis of ^{1}H and ^{13}C NMR

data revealed that **14** is an oxygenated derivative of sapelin E acetate.[12] The most significant differences were the presence of two CH$_2$ group signals instead of $\Delta^{1(2)}$ double-bond signals. The HMBC correlation from Me-19 to C-1 (δ_C 38.8) as well as the ^{1}H–^{1}H COSY cross-peaks of H$_2$-1/H$_2$-2 further confirmed the above inference. Thus, the structure of **14** was established as dihydrosapelin E acetate.

Fourteen known compounds, dihydroniloticin (**15**),[13] mesendanin T (**16**),[14] nimonol (**17**),[15] 12α-acetoxy-7-deacetylazadiron (**18**),[16] turraflorin A (**19**),[17] 1α-acetoxy-3α-propanoyloxyvilasinin (**20**),[18] mesendanin U (**21**),[14] mzikonone (**22**),[15] 11-*epi*-toonacilin (**23**),[6] 23-hydroxytoonacilide (**24**),[19] turrapubesin E (**25**),[7] azadirachtin (**26**),[10] turrapubesic acid B (**27**),[7] and hispidol B (**28**),[20] were identified by analysis of their spectroscopic data and comparison with the literature values.

All compounds were evaluated for their cytotoxic activity against HL-60 cells and their inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 cells. However, none of the compounds were found to be cytotoxic against HL-60 cells (IC$_{50}$ < 10 μM). In an anti-inflammatory assay, compounds **12**, **16**, **18**, **19**, and **21** exhibited an inhibitory effect on LPS-stimulated NO production in RAW 264.7 cells, whereas the other compounds were inactive in this assay (IC$_{50}$ value >20 μM). At a concentration of 50 μM, none of the tested compounds had any potential cytotoxicity toward RAW264.7 cells. Selected compounds were evaluated for their inhibitory activity against brine shrimp (*Artemia salina*) larvae by the microwell method. Compounds **2**, **11**, **18**, and **26** exhibited inhibitory activity against brine shrimp larvae at 100 ppm, with the corrected mortalities ranging from 81.7% to 100% (Table 7).

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were recorded on a Shimadzu UV-2401A. CD spectra were recorded with an Applied Photophysics Chirascan spectrometer. IR spectra were determined on a Bruker Tensor-27 infrared spectrophotometer with KBr disks. ^{1}H and ^{13}C NMR and 2D-NMR spectra were recorded on Bruker AM-400, Bruker DRX-500, and Bruker Avance III 600 spectrometers using TMS as an internal standard. Bruker HCT/E Squire and Waters Autospec Premier P776 mass spectrometers were used to measure ESIMS and HREIMS, respectively. Column chromatography was performed on silica gel (90−150 μm, Qingdao Marine Chemical Company, Qingdao, People's Republic of China), Sephadex LH-20 (40−70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-C$_{18}$ gel (40−63 μm, Merck, Darmstadt, Germany). TLC spots were visualized under UV light and by dipping into 5% H$_2$SO$_4$ in EtOH followed by heating.

Plant Material. The dried twigs of *T. pubescens* were collected in Dongfang, Hainan Province, China, in March 2012 and were identified by one of the authors (G.-H.T.). A voucher specimen (H201203001) was deposited at the Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried, powdered twigs of *T. pubescens* (12.0 kg) were extracted with MeOH (3 × 25 L) under reflux three times (4, 3, and 3 h), respectively. The combined MeOH extracts were concentrated under vacuum to give the crude residue (900 g), which was suspended in water and then partitioned with EtOAc. The EtOAc portion (200 g) was subjected to passage over a silica gel column, eluted with a gradient of petroleum ether−acetone (from 1:0 to 0:1), to yield six fractions (1−6). Fr. 3 (60 g) was then separated over a MCI-gel column (MeOH−H$_2$O from 3:7 to 10:0) to obtain four fractions (3A−3D). Fr. 3A (13 g) was chromatographed

Table 5. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds 12−14 in CDCl$_3$

position	12[a] δ$_H$ (J in Hz)	12 δ$_C$, type	13[b] δ$_H$ (J in Hz)	13 δ$_C$, type	14[b] δ$_H$ (J in Hz)	14 δ$_C$, type
1α	7.12, d (10.2)	158.5, d	1.52, m	38.5, t	1.50, m	38.8, t
1β			1.87, m		1.91, m	
2α	5.81, d (10.2)	125.7, d	2.44, m	33.9, t	2.42, m	34.0, t
2β			2.53, m		2.59, m	
3		205.5, s		217.4, s		216.8, s
4		44.4, s		46.9, s		46.9, s
5	2.37, dd (12.5, 2.8)	44.7, d	2.08, m	46.5, d	1.85, m	48.2, d
6α	1.83, m	24.4, t	1.84, m	24.7, t	1.86, m	24.2, t
6β	1.87, m		1.79, m		1.70, m	
7	3.97, t (2.9)	71.7, d	3.96, s	72.0, d	5.20, s	75.1, d
8		45.0, s		44.0, s		41.9, s
9	2.19, m	36.8, d	2.04, m	41.0, d	2.00, m	42.8, d
10		40.4, s		37.1, s		36.9, s
11α	1.96, m	16.6, t	1.75, m	16.2, t	1.66, m	16.7, t
11β	1.72, m		1.60, m		1.54, m	
12α	1.69, m	32.7, t	1.85, m	32.7, t	1.83, m	34.0, t
12β	1.57, m		1.50, m		1.54, m	
13		46.8, s		47.0, s		46.3, s
14		161.7, s		161.9, s		159.3, s
15	5.49, s	120.1, d	5.48, s	119.4, d	5.27, s	118.8, d
16α	2.15, m	35.3, t	2.19, m	34.7, t	1.98, m	35.0, t
16β			2.13, m		2.24, m	
17	1.99, m	53.0, d	1.75, m	57.7, d	1.87, m	54.2, d
18	1.00, s	20.2, q	1.06, s	19.5, q	0.97, s	19.6, q
19	1.15, s	19.1, q	1.01, s	15.0, q	1.02, s	15.1, q
20	2.19, m	45.3, d	2.38, m	46.6, d	1.89, m	36.3, d
21α	4.81, d (4.1)	104.4, d	4.86, d (3.0)	109.1, d	3.61, dd (13.0, 2.0)	64.3, t
21β					3.48, dt (13.0, 2.0)	
22α	2.00, m	31.9, t	1.36, m	34.4, t	1.86, m	24.2, t
22β	1.62, m		2.06, m		1.70, m	
23	3.84, m	78.8, d	3.81, m	76.9, d	3.80, dt (9.0, 2.6)	68.1, d
24	2.71, d (8.0)	67.8, d	2.71, d (7.4)	65.2, d	3.42, d (9.0)	80.7, d
25		57.5, s		57.1, s		76.1, s
26	1.26, s	19.6, q	1.31, s	19.4, q	1.30, s	26.3, q
27	1.30, s	25.1, q	1.33, s	24.9, q	1.15, s	22.4, q
28	1.14, s	27.3, q	1.05, s	21.1, q	1.00, s	25.8, q
29	1.07, s	21.7, q	1.10, s	26.2, q	1.03, s	21.0, q
30	1.11, s	27.8, q	1.10, s	27.1, q	1.14, s	26.9, q
OMe-21	3.36, s	54.4, q	3.38, s	55.5, q		
OAc-7						170.3, s
					1.95, s	21.2, s

[a] ^{1}H and ^{13}C NMR spectroscopic data were recorded at 600 and 150 MHz. [b] ^{1}H and ^{13}C NMR spectroscopic data were recorded at 500 and 125 MHz.

Table 6. Inhibition of Nitric Oxide Production of Compounds 1−28[a]

compound	anti-inflammation (IC$_{50}$ μM) NO
12	17.4
16	13.2
18	11.9
19	15.4
21	18.5
MG-132	0.17

[a] Compounds 1−11, 13−15, 17, 20, and 22−28 were inactive for inhibition of nitric oxide production (IC$_{50}$ > 20 μM). MG-132 is a positive control.

over a C$_{18}$ silica gel column, eluted with a gradient of MeOH−H$_2$O (60:40, 65:35, and 70:30), to afford five subfractions (3A1−3A5). Fr. 3A1 (2.5 g) was purified by Sephadex LH-20 (MeOH) and then chromatographed on a silica gel column (CHCl$_3$−Me$_2$CO, 9:1) to obtain 1 (206 mg), 5 (15 mg), 18 (46.2 mg), and 22 (16.4 mg). Compounds 23 (73.0 mg), 19 (2.1 mg), and 8 (8.6 mg) were isolated from Fr. 3A2 (800 mg) by repeated silica gel column chromatography, eluted with a gradient of CHCl$_3$−Me$_2$CO (from 100:2 to 100:10). Fr. 3B (2.5 g) was separated by Sephadex LH-20 (MeOH−CHCl$_3$, 1:1) to obtain 4 (7.5 mg) and a major fraction (Fr. 3B1). Fr. 3B1 (800 mg) was separated over semipreparative HPLC (MeOH−H$_2$O, 6:4) to give 2 (38.6 mg), 3 (55 mg), 21 (5.5 mg), and 7 (100 mg). Fr. 3C (8 g) was chromatographed on a silica gel column, eluted with a gradient of petroleum ether−EtOAc (9:1 to 5:5), to yield 17 (6.6 mg), 16 (7.8 mg), and subfractions 3C1 (60 mg) and 3C2 (30 mg). Subfractions 3C1 and 3C2 were further purified by semipreparative HPLC (MeOH−H$_2$O, 4:6) to afford 6 (6.6 mg), 9 (2.1 mg), and 20 (6.2

Table 7. Corrected Mortality of Selected Compounds against Brine Shrimp[a]

	corrected mortality (%)		
compound	100 ppm	50 ppm	10 ppm
1	54.7	0.8	0
2	81.7	61.8	60.8
3	63.3	25.8	0.5
10	71.3	0	0
11	100.0	5.3	0
14	69.8	6.7	0
18	85.0	83.5	20.6
20	75.6	36.3	0
22	76.4	36.9	0
26	81.7	59.6	12.5

[a]Corrected mortality = $(M_t - M_c)/(1 - M_c) \times 100\%$. The mean mortality of control group was 4.7%. M_t: mortality of treatment group, M_c: mortality of control group. Compounds **5**, **8**, **19**, **23**, **24**, and **25** showed no corrected mortality at 100 ppm (corrected mortality <50%).

mg). Fr. 4 (46 g) was chromatographed over a MCI-gel column (MeOH−H$_2$O from 5:5 to 10:0) to obtain five fractions (4A−4E). Fr. 4A (1.5 g) was purified using Sephadex LH-20 (MeOH) to furnish **11** (30 mg), **12** (3.1 mg), and major fraction 4A1 (800 mg), which was separated by semipreparative HPLC using MeOH−H$_2$O (46:54) to give **13** (6.5 mg), **15** (9 mg), and **28** (5.2 mg). Fr. 4B (8 g) was chromatographed over a silica gel column, eluted with a gradient of CHCl$_3$−MeOH (from 100:1 to 100:9), to afford **26** (25 mg), **14** (9 mg), **10** (250 mg), and **24** (61 mg). Fr. 4C (5 g) was chromatographed on a C$_{18}$ silica gel column, eluted with a gradient of MeOH−H$_2$O (60:40, 65:45, and 70:30), to afford **25** (27 mg) and **27** (5.0 mg).

Turrapubin A (1): white, amorphous power; $[\alpha]^{25}_D$ −6.9 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.89) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (+19.4), 289 (−7.1); IR (KBr) ν_{max} 3441, 2981, 1735, 1433, 1376, 1242, 1044, 1027 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive ESIMS *m/z* 637 [M + Na]$^+$; HREIMS *m/z* 614.2733 [M]$^+$ (calcd for C$_{33}$H$_{42}$O$_{11}$, 614.2727).

Turrapubin B (2): white, amorphous power; $[\alpha]^{25}_D$ +0.6 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.85) nm; IR (KBr) ν_{max} 3436, 2981, 1741, 1377, 1240, 1169, 1028 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive ESIMS *m/z* 665 [M + Na]$^+$; HREIMS *m/z* 642.3043 [M]$^+$ (calcd for C$_{35}$H$_{46}$O$_{11}$, 642.3040).

Turrapubin C (3): white, amorphous power; $[\alpha]^{25}_D$ −1.6 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.84) nm; IR (KBr) ν_{max} 3435, 2977, 1741, 1376, 1240, 1171, 1028 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive ESIMS *m/z* 679 [M + Na]$^+$; HREIMS *m/z* 656.3179 [M]$^+$ (calcd for C$_{36}$H$_{48}$O$_{11}$, 656.3197).

Turrapubin D (4): white, amorphous power; $[\alpha]^{25}_D$ −16.9 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.03) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 227 (+3.8), 299 (+0.89); IR (KBr) ν_{max} 3442, 2955, 1738, 1714, 1373, 1236, 1032 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 535 [M + Na]$^+$; HREIMS *m/z* 512.2437 [M]$^+$ (calcd for C$_{29}$H$_{36}$O$_8$, 512.2410).

Turrapubin E (5): white, amorphous power; $[\alpha]^{25}_D$ +62.3 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.20), 235 (4.12); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (+5.5), 237 (−17.0), 330 (+11.6) nm; IR (KBr) ν_{max} 3443, 1737, 1675, 1370, 1237, 1034 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 535 [M + Na]$^+$; HREIMS *m/z* 512.2406 [M]$^+$ (calcd for C$_{29}$H$_{36}$O$_8$, 512.2410).

Turrapubin F (6): white, amorphous power; $[\alpha]^{25}_D$ +147.9 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.24) 235 (4.18); IR (KBr) ν_{max} 3440, 2980, 1737, 1679, 1225, 1037 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 605 [M + Na]$^+$; HREIMS *m/z* 582.2829 [M]$^+$ (calcd for C$_{33}$H$_{42}$O$_9$, 582.2829).

Turrapubin G (7): white, amorphous power; $[\alpha]^{25}_D$ +90.0 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.05), 233 (3.97) nm; IR (KBr) ν_{max} 3442, 2975, 1751, 1737, 1680, 1225, 1030 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 619 [M + Na]$^+$; HREIMS *m/z* 596.2965 [M]$^+$ (calcd for C$_{34}$H$_{44}$O$_9$, 596.2985).

Turrapubin H (8): white, amorphous power; $[\alpha]^{25}_D$ +60.4 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.13), 235 (3.99) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (+17.3), 2376 (−8.2), 331 (+7.0); IR (KBr) ν_{max} 3441, 1738, 1716, 1631, 1250, 1127, 1021 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 637 [M + Na]$^+$; HREIMS *m/z* 614.2713 [M]$^+$ (calcd for C$_{33}$H$_{42}$O$_{11}$, 614.2727).

Turrapubin I (9): white, amorphous power; $[\alpha]^{25}_D$ −7.0 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.93) nm; IR (KBr) ν_{max} 3440, 2939, 1731, 1376, 1247, 1050 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 593 [M + Na]$^+$; HREIMS *m/z* 570.3198 [M]$^+$ (calcd for C$_{33}$H$_{46}$O$_8$, 570.3193).

Turrapubin J (10): white, amorphous power; $[\alpha]^{25}_D$ −32.1 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 251 (3.18), 211 (4.04) nm; IR (KBr) ν_{max} 3424, 2956, 1742, 1626, 1267, 1162, 1128, 1086, 1037 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 4; positive ESIMS *m/z* 757 [M + Na]$^+$; HREIMS *m/z* 734.2795 [M]$^+$ (calcd for C$_{36}$H$_{46}$O$_{16}$, 734.2786).

Turrapubin K (11): white, amorphous power; $[\alpha]^{25}_D$ −56.2 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.09) nm; IR (KBr) ν_{max} 3442, 2955, 1737, 1620, 1270, 1163, 1059, 1037 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 4; positive ESIMS *m/z* 757 [M + Na]$^+$; HREIMS *m/z* 734.2783 [M]$^+$ (calcd for C$_{36}$H$_{46}$O$_{16}$, 734.2786).

7-Deacetylbruceajavanin B (12): white, amorphous power; $[\alpha]^{25}_D$ +23.9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.17), 209 (4.17) nm; IR (KBr) ν_{max} 3433, 2938, 1669, 1459, 1385, 1121, 1060, 1032, 968 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 5; positive ESIMS *m/z* 521 [M + Na]$^+$; HREIMS *m/z* 498.3358 [M]$^+$ (calcd for C$_{31}$H$_{46}$O$_5$, 498.3345).

7-Deacetyl-21α-methoxydihydrobruceajavanin B (13): white, amorphous power; $[\alpha]^{25}_D$ −71.4 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.77) nm; IR (KBr) ν_{max} 3441, 2927, 1702, 1461, 1382, 1104, 1063, 1051, 1033 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 5; positive ESIMS *m/z* 523 [M + Na]$^+$; HREIMS *m/z* 500.3488 [M]$^+$ (calcd for C$_{31}$H$_{48}$O$_5$, 500.3502).

Dihydrosapelin E acetate (14): white, amorphous power; $[\alpha]^{25}_D$ −29.3 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.54) nm; IR (KBr) ν_{max} 3442, 2936, 1735, 1709, 1461, 1378, 1247, 1033 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 5; positive ESIMS *m/z* 553 [M + Na]$^+$; HREIMS *m/z* 530.3572 [M]$^+$ (calcd for C$_{32}$H$_{50}$O$_6$, 530.3607).

Cytotoxicity Assays. All isolates were evaluated for the cytotoxicities against HL-60 (myeloid leukemia) by using the MTT assay.[21] Cytotoxicity evaluations were performed according to a previously described protocol.[22]

Inhibition of Nitric Oxide Production Assay. Inhibition of NO production was determined in LPS-stimulated RAW 264.7 macrophage cell lines. Murine monocytic RAW 264.7 macrophages were dispensed into 96-well plates (2 × 10^5 cells/well) containing RPMI 1640 medium (Hyclone, UT, USA) with 10% FBS under a humidified atmosphere of 5% CO$_2$ at 37 °C. After 24 h preincubation, cells were treated with serial dilutions of all isolated compounds with the maximum concentration of 25 μM in the presence of 1 μg/mL LPS for 18 h. Each compound was dissolved in DMSO and further diluted in medium to produce different concentrations. NO production in each well was assessed by adding 100 μL of Griess reagents A and B to 100 μL of each supernatant from LPS or the compound-treated cells in triplicate. After a 5 min incubation, the absorbance was measured at 570 nm with a 2104 Envision multilabel plate reader (Perkin−Elmer Life Sciences, Inc., Boston, MA, USA). Cytotoxicity was determined by the MTT assay.[21] MG-132 was used as a positive control. The assay was performed as described previously.[23]

Brine Shrimp Lethality Bioassay. Inhibitory activity for brine shrimp (*Artemia salina*) larvae by the microwell method was performed according to the previously described protocol.[24]

■ ASSOCIATED CONTENT

S Supporting Information

This material (1D- and 2D-NMR, ESIMS, HREIMS, UV, and IR spectra of new compounds (**1**−**14**) and the CD spectra of compounds **1**, **4**, **5**, and **8**) is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(H.-M. Hua) Tel: +86-24-23986465. Fax: +86-24-23986465. E-mail: huimhua@163.com. (H.-P. He) Tel/Fax: +86-871-65223070. E-mail: hehongping@mail.kib.ac.cn. (X.-J. Hao) E-mail: haoxj@mail.kib.ac.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) (a) Fang, X.; Di, Y. T.; Hao, X. J. *Curr. Org. Chem.* **2011**, *15*, 1363−1391. (b) Tan, Q. G.; Luo, X. D. *Chem. Rev.* **2011**, *112*, 7437−7522.

(2) Peng, H.; Mabberley, D. J. *Flora of China*; Science Press: Beijing, 2008; Vol. *11*, pp 117−118.

(3) Editorial Committee of the Administration Bureau of Traditional Chinese Medicine. In *Chinese Materia Medica (Zhonghua Benchao)*; Shanghai Science and Technology Press: Shanghai, 1999; Vol. *3*, pp 30−49.

(4) (a) Bentley, M. D.; Gaul, F.; Rajab, M. S.; Hassanali, A. *J. Nat. Prod.* **1992**, *55*, 84−87. (b) Adul, G. O.; Bentley, M. D.; Benson, B. W.; Huang, F. Y.; Gelbaum, L.; Hassanali, A. *J. Nat. Prod.* **1993**, *56*, 1414−1417. (c) Wang, X. N.; Fan, C. Q.; Yue, J. M. *Steroids* **2006**, *71*, 720−724. (d) Wang, X. N.; Yin, S.; Fan, C. Q.; Wang, F. D.; Lin, L. P.; Ding, J.; Yue, J. M. *Org. Lett.* **2006**, *8*, 3845−3848. (e) Wang, X. N.; Fan, C. Q.; Yin, S.; Lin, L. P.; Ding, J.; Yue, J. M. *Helv. Chim. Acta* **2008**, *91*, 510−519.

(5) (a) Fang, X.; Di, Y. T.; He, H. P.; Liu, H. Y.; Zhang, Z.; Ren, Y. L.; Gao, Z. L.; Gao, S.; Hao, X. J. *Org. Lett.* **2008**, *10*, 1905−1908. (b) Ning, J.; Di, Y. T.; Fang, X.; He, H. P.; Wang, Y. Y.; Li, Y.; Li, S. L.; Hao, X. J. *J. Nat. Prod.* **2010**, *73*, 1327−1331. (c) Ning, J.; Di, Y. T.; Wang, Y. Y.; He, H. P.; Fang, X.; Li, Y.; Li, S. L.; Hao, X. J. *Planta Med.* **2010**, *76*, 1907−1910. (d) Yin, J. L.; Di, Y. T.; Fang, X.; Liu, E. D.; Liu, H. Y.; He, H. P.; Li, S. L.; Li, S. F.; Hao, X. J. *Tetrahedron Lett.* **2011**, *52*, 3083−3085. (e) Yuan, C. M.; Zhang, Y.; Tang, G. H.; Li, S. L.; Di, Y. T.; Hou, L.; Cai, J. Y.; Hua, H. M.; He, H. P.; Hao, X. J. *Chem. Asian J.* **2012**, *7*, 2024−2027. (f) Cai, J. Y.; Zhang, Y.; Luo, S. H.; Chen, D. Z.; Tang, G. H.; Yuan, C. M.; Di, Y. T.; Li, S. H.; Hao, X. J.; He, H. P. *Org. Lett.* **2012**, *14*, 2524−2527. (g) Yuan, C. M.; Zhang, Y.; Tang, G. H.; Di, Y. T.; Cao, M. M.; Wang, X. Y.; Zuo, G. Y.; Li, S. L.; Hua, H. M.; He, H. P.; Hao, X. J. *J. Nat. Prod.* **2013**, *76*, 327−333.

(6) Mulholland, D. A.; Monkhe, T. V.; Coombes, P. H.; Rajab, M. S. *Phytochemistry* **1998**, *49*, 2585−2590.

(7) Wang, X. N.; Yin, S.; Fan, C. Q.; Lin, L. P.; Ding, J.; Yue, J. M. *Tetrahedron* **2007**, *63*, 8234−8241.

(8) Dong, S. H.; Zhang, C. R.; He, X. F.; Liu, H. B.; Wu, Y.; Yue, J. M. *J. Nat. Prod.* **2010**, *73*, 1344−1349.

(9) (a) Kumar, C. S. S. R.; Srinivas, M.; Yakkundi, S. *Phytochemistry* **1996**, *43*, 451−455. (b) Tan, Q. G.; Li, X. N.; Chen, H.; Feng, T.; Cai, X. H.; Luo, X. D. *J. Nat. Prod.* **2010**, *73*, 693−697.

(10) Klenk, A.; Bokel, M.; Kraus, W. *J. Chem. Soc., Chem. Commun.* **1986**, 523−524.

(11) (a) Kitagawa, I.; Mahmud, T.; Simanjuntak, P.; Hori, K.; Uji, T.; Shibuya, H. *Chem. Pharm. Bull.* **1994**, *42*, 1416−1421. (b) Dong, S. H.; Liu, J.; Ge, Y. Z.; Dong, L.; Xu, C. H.; Ding, J.; Yue, J. M. *Phytochemistry* **2013**, *85*, 175−184.

(12) Mitsui, K.; Saito, H.; Yamamura, R.; Fukaya, H.; Hitotsuyanagi, Y.; Takeya, K. *Chem. Pharm. Bull.* **2007**, *55*, 1442−1447.

(13) Itokawa, H.; Kishi, E.; Morita, H.; Takeya, K. *Chem. Pharm. Bull.* **1992**, *40*, 1053−1055.

(14) Dong, S. H.; He, X. F.; Dong, L.; Wu, Y.; Yue, J. M. *Helv. Chim. Acta* **2012**, *95*, 286−300.

(15) Rajab, M. S.; Bentley, M. D.; Hassanali, A.; Chapya, A. *Phytochemistry* **1988**, *27*, 2353−2355.

(16) Odero, O. J. H.; Ndung'u, M.; Hassanali, A. *J. Kenya Chem. Soc.* **2008**, *5*, 43−49.

(17) Mcfarland, K.; Mulholland, D. A.; Fraser, L. A. *Phytochemistry* **2004**, *65*, 2031−2037.

(18) Ndung'u, M. W.; Kaoneka, B.; Hassanali, A.; Lwande, W.; Hooper, A. M.; Tayman, F.; Zerbe, O.; Torto, B. *J. Agric. Food Chem.* **2004**, *52*, 5027−5031.

(19) Zhang, F.; Wang, J. S.; Gu, Y. C.; Kong, L. Y. *J. Nat. Prod.* **2012**, *75*, 538−546.

(20) Jolad, S. D.; Hoffmann, J. J.; Schram, K. H.; Cole, J. R.; Tempesta, M. S.; Bates, R. B. *J. Org. Chem.* **1981**, *46*, 4085−4088.

(21) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.

(22) Guo, L. L.; He, H. P.; Di, Y. T.; Li, S. F.; Cheng, Y. Y.; Yang, W.; Li, Y.; Yu, J. P.; Zhang, Y.; Hao, X. J. *Phytochemistry* **2012**, *74*, 140−145.

(23) Zhou, X. J.; Chen, X. L.; Li, X. S.; Su, J.; He, J. B.; Wang, Y. H.; Li, Y.; Cheng, Y. X. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 373−376.

(24) Cao, M. M.; Zhang, Y.; He, H. P.; Li, S. F.; Huang, S. D.; Chen, D. Z.; Tang, G. H.; Li, S. L.; Di, Y. T.; Hao, X. J. *J. Nat. Prod.* **2012**, *75*, 1076−1082.