Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron 63 (2007) 8234–8241
Tetrahedron

Eight new limonoids from *Turraea pubescens*

Xiao-Ning Wang, Sheng Yin, Cheng-Qi Fan, Li-Ping Lin, Jian Ding and Jian-Min Yue*

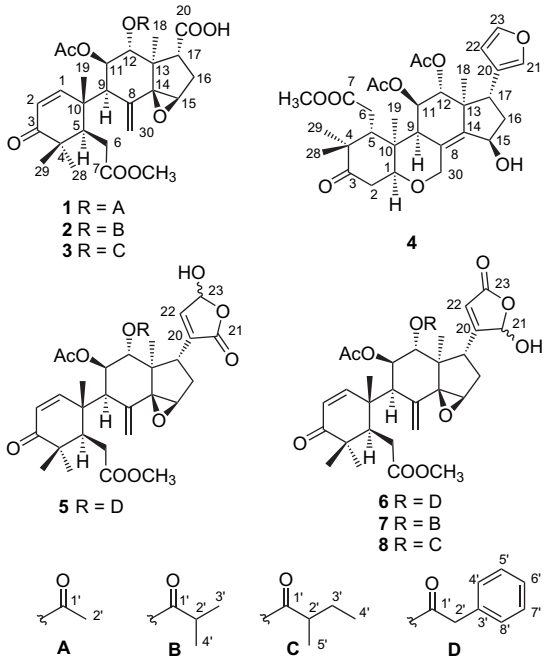
State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China

Received 1 February 2007; revised 27 April 2007; accepted 28 May 2007
Available online 2 June 2007

Abstract—Eight new ring B-*seco* limonoids, turrapubesic acids A–C (**1**–**3**) and turrapubesins C–G (**4**–**8**), along with turraflorin E and isoazadironolide were isolated from the twigs and leaves of *Turraea pubescens*. Turrapubesic acids A–C (**1**–**3**) are a group of ring B-*seco* limonoid 17-carboxylic acids with a new C_{23} skeleton, and turrapubesin C (**4**) incorporates an unprecedented 1,30-oxygen bridge. The structures including absolute stereochemistry of **1**–**8** were established on the basis of extensive NMR spectroscopic analysis and CD study. The cytotoxicity of the isolates against the P-388 and A-549 cells was evaluated.

1. Introduction

Plants of the Meliaceae family have been attracting considerable interest because of the diversified structures and the significant biological activities of the limonoids.[1] *Turraea pubescens* Hellen (Meliaceae) is a wild shrub distributed mainly in the tropical and subtropical areas of South and Southeast Asia and Western Australia.[2] The twigs and leaves of this plant have been applied to treat dysentery, pharyngolaryngitis, and traumatic hemorrhage in the Chinese folklore medicine.[2] A number of protolimonoids, limonoids, and other kinds of metabolites have been isolated from plants of the same genus.[1c,3] Previously, we reported the isolation and characterization of three new pregnane steroids[4] and two unusual ring B-*seco* limonoids[5] from *T. pubescens* collected in Hainan Island of the People's Republic of China. In the present research, eight new limonoids, turrapubesic acids A–C (**1**–**3**) and turrapubesins C–G (**4**–**8**), along with turraflorin E (**9**),[3d] and isoazadironolide (**10**)[6] were isolated from the twigs and leaves of *T. pubescens*. Turrapubesic acids A–C (**1**–**3**) are a group of ring B-*seco* limonoid 17-carboxylic acids with a new C_{23} skeleton, while turrapubesin C (**4**) incorporates an unprecedented 1,30-oxygen bridge. Herein, we report the isolation, structure elucidation, and cytotoxicity evaluation of **1**–**10**.



2. Results and discussion

2.1. Turrapubesic acids A–C (1–3)

Turrapubesic acid A (**1**) was obtained as a white amorphous solid. The HREIMS exhibited a molecular ion peak at *m*/*z* 532.2306 (calcd 532.2308) corresponding to the molecular

Keywords: *Turraea pubescens*; Limonoids; Turrapubesic acids; Turrapubesins; Absolute stereochemistry.
* Corresponding author. Tel./fax: +86 21 50806718; e-mail: jmyue@mail.shcnc.ac.cn

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.05.107

formula $C_{28}H_{36}O_{10}$, which was consistent with the NMR data, indicating 11 degrees of unsaturation. The IR spectrum exhibited absorption bands for hydroxyls (3433 cm^{-1}), carbonyls (1738 cm^{-1}), and double bonds (1680 cm^{-1}). The UV absorption band at 233 nm was suggestive of an α,β-unsaturated ketone group. The ^{13}C and DEPT spectra displayed 28 carbon resonances comprising seven methyls, two sp^3 methylenes, six sp^3 methines (three oxygenated at δ_C 74.2, 70.8, and 59.6), five carbonyls (one ketone and four ester and/or carboxyl carbonyls), two double bonds, and four quaternary sp^3 carbons (one oxygenated at δ_C 70.8). The seven methyls were consistent with four tertiary methyls [δ_H 0.97 (s, H_3-18), 0.93 (s, H_3-19), 0.97 (s, H_3-28), and 1.07 (s, H_3-29); δ_C 12.9, 21.2, 22.9, and 22.6], one methoxy [δ_H 3.66 (s); δ_C 52.1], and two acetyls [δ_H 1.88 (s) and 1.91 (s); δ_C 20.6, 170.0, 20.5, and 169.7] as deduced from the ^1H and ^{13}C NMR data (Tables 1 and 2). The proton signals at δ_H 7.37 (d, J=10.4 Hz, H-1) and 6.12 (d, J=10.4 Hz, H-2) together with carbon signals at δ_C 152.2 (C-1), 125.7 (C-2), and 204.0 (C-3) indicated the presence of an α,β-unsaturated ketone. Furthermore, the two non-equivalent protons at δ_H 5.31 (H-30a) and 5.23 (H-30b) and the carbons at δ_C 135.8 (C-8) and 121.5 (C-30) revealed an exocyclic $\Delta^{8(30)}$ double bond. The ^1H and ^{13}C NMR data, in combination with the HMBC correlation analysis (Fig. 1) furnished the planar structure of **1**, which is closely related to that of 11-*epi*-23-hydroxytoonacilide (**11**) except for the C-17 side chain.[3a] In the HMBC spectrum, one carbon resonance at δ_C 177.3 correlated with H-16α and H-17 was assigned to the C-20 carboxyl group, which is consistent with the C-20 chemical shift value and molecular formula.[7]

The relative configuration of **1** was established by ROESY experiment (Fig. 2), in which, the correlations of H_3-18/H-11, H_3-18/H-16α, H-11/H-9, H-12/H-17, H-30a/H-15, and H-30b/H-9 were consistent with the configurations of rings C and D, and this was supported by the large coupling constants of $J_{9,11}$=7.4 Hz and $J_{11,12}$=10.8 Hz for the typical 11β,12α-substituted ring B-*seco* limonoids.[8] Furthermore, ROESY correlations of H-5/H_3-28, H-6β/H_3-19, H-6β/H_3-29, and H_3-19/H_3-29 furnished the configuration of ring A as depicted. Although the C-9–C-10 bond could rotate to certain extent, the steric bulk of rings A and C made it fairly fixed as judged by the strong NOE correlations of H-12/H-1, H-30b/H_3-19, and H-9/OMe-7.

The absolute configuration of **1** was determined by applying the CD exciton chirality method.[9] The CD of **1** (Fig. 3) showed negative split between the two chromophores of the α,β-unsaturated ketone (234 nm, $\Delta\varepsilon$ −3.31, $\pi\rightarrow\pi^*$ transition)[10] and the $\Delta^{8(30)}$ double bond (200 nm, $\Delta\varepsilon$ +2.36, $\pi\rightarrow\pi^*$ transition),[11] indicating that the transition dipole moments of the two chromophores were oriented in a counterclockwise manner (Fig. 3), and the absolute configuration of the nine chiral centers in **1** was thus determined as 5*R*, 9*R*, 10*S*, 11*R*, 12*R*, 13*R*, 14*S*, 15*R*, and 17*R*. The positive Cotton effect at 328 nm ($\Delta\varepsilon$ +3.48) due to the $n\rightarrow\pi^*$ transition of the carbonyl groups of **1** was in accordance with those of turrapubesins A (**13**) and B (**14**),[5] which also supported the absolute configuration assigned for **1**.

Turrapubesic acid B (**2**) was obtained as colorless needles. The HRESIMS displayed a pseudo molecular ion at *m/z* 583.2539 $[M+Na]^+$ consistent with the molecular formula $C_{30}H_{40}O_{10}$ (calcd for $C_{30}H_{40}O_{10}Na$, 583.2519). The IR and UV spectra exhibited absorption bands similar to those of **1**. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) also resembled those of **1** closely, except for the absence of the C-12 acetoxy group, which was replaced by an isobutanoyloxy moiety as judged by the proton resonances at δ_H 2.38 (m, H-2′), 1.06 (d, J=7.0 Hz, H_3-3′), and 1.04 (d, J=7.0 Hz, H_3-4′), and the carbon signals at δ_C 175.6 (C-1′), 33.8 (C-2′), 18.2 (C-3′), and 18.8 (C-4′). The HMBC correlations of H-2′/C-1′, C-3′, and C-4′; H_3-3′/C-1′ and C-2′; and H_3-4′/C-1′ and C-2′ confirmed the presence of the isobutanoyloxy group. The key HMBC correlation between H-12 (δ_H 5.75, d, J=10.7 Hz) and C-1′ indicated that the isobutanoyloxy was attached at C-12. Accordingly, the planar structure of turrapubesic acid B (**2**) was established as the isobutanoate analogue of **1**.

Turrapubesic acid C (**3**) was obtained as colorless crystals. A molecular formula of $C_{31}H_{42}O_{10}$ was assigned for **3** from the molecular ion peak at *m/z* 574.2791 (calcd 574.2778) in the HREIMS. Analysis of the IR and UV, together with 1D and 2D NMR data of **3** (Tables 1 and 2) indicated that it shared the identical limonoid skeleton with **1** and **2**. The only difference in **3** was the C-12 ester group, which was identified as a 2-methylbutanoate moiety on the basis of ^1H NMR and ^{13}C NMR data (Tables 1 and 2). The HMBC correlations of H-2′/C-1′, C-3′, C-4′, and C-5′; H_2-3′/C-1′, C-2′, C-4′, and C-5′; H_3-4′/C-2′ and C-3′; H_3-5′/C-1′, C-2′, and C-3′; and H-12/C-1′ confirmed the presence of 2-methylbutanoate and its attachment to C-12. The planar structure of **3** was thus determined to be a 2-methylbutanoate analogue of **1** and **2**.

The relative configurations of turrapubesic acids B (**2**) and C (**3**) were assigned to be the same as that of **1** based on their almost superimposable ^1H and ^{13}C NMR data and similar NOE correlation patterns to those of **1**. The absolute configurations of **2** and **3** were identical to that of **1**, as determined by their similar Cotton effects in the CD spectra (Fig. 3).[9]

Turrapubesic acids A–C (**1**–**3**) are a group of ring B-*seco* limonoids containing a new C_{23} skeleton with a 17-carboxylic acid moiety, which were derived by degradation of the side chain of the biosynthetic precursor euphol or tirucallol.[1,7] To the best of our knowledge, this is the second finding of limonoid derivatives with a carboxyl at C-17.[7]

2.2. Turrapubesin C (4)

Turrapubesin C (**4**) was isolated as an amorphous solid. The HRESIMS showed a pseudo molecular ion peak at *m/z* 595.2512 $[M+Na]^+$ (calcd for $C_{31}H_{40}O_{10}Na$, 595.2519) corresponding to the molecular formula $C_{31}H_{40}O_{10}$. The IR spectrum exhibited absorption bands for hydroxyls (3437 cm^{-1}), carbonyl groups (1747 and 1718 cm^{-1}), and double bonds (1637 cm^{-1}). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) revealed four tertiary methyls [δ_H/δ_C 0.91/16.5 (Me-18), 1.18/19.9 (Me-19), 1.07/23.5 (Me-28), and 0.95/24.8 (Me-29)], one carbomethoxy group [δ_H 3.79; δ_C 52.3 and 175.9 (C-7)], two acetyls [δ_H 1.64 (s) and 2.02 (s); δ_C 20.3, 170.8, 21.2, and 170.3], and a β-furyl ring (Tables 1 and 2). Analysis of the ^1H–^1H COSY, HSQC, and HMBC spectra (Fig. 4) indicated that the NMR data of **4**

Table 1. [1]H NMR spectroscopic data for compounds **1–8**[a]

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	7.37 (d, 10.4)	7.42 (d, 10.6)	7.39 (d, 10.6)	3.63 (dd, 3.8, 2.2)	7.38 (d, 10.5)	7.34 (d, 10.8)	7.34 (d, 10.6)	7.36 (d, 10.6)
2	6.12 (d, 10.4)	6.16 (d, 10.6)	6.15 (d, 10.6)	α 3.00 (dd, 15.1, 3.8) β 2.34 (dd, 15.1, 2.2)	6.13 (d, 10.5)	6.11 (d, 10.8)	6.12 (d, 10.6)	6.14 (d, 10.6)
5	2.93 (dd, 7.8, 2.5)	2.93 (dd, 7.6, 2.6)	2.89 (dd, 7.8, 2.3)	2.87 (d, 9.6)	2.81 (br d, 7.5)	2.89 (m)	2.94 (m)	2.93 (dd, 7.6, 2.5)
6α	2.30 (dd, 16.8, 2.5)	2.36 (m)	2.32 (m)	2.68 (dd, 18.0, 9.6)	2.26 (m)	2.30 (m)	2.31 (m)	2.33 (dd, 16.9, 2.5)
6β	2.43 (dd, 16.8, 7.8)	2.46 (dd, 16.9, 7.7)	2.44 (dd, 16.8, 7.8)	3.18 (d, 18.0)	2.40 (dd, 16.7, 7.7)	2.43 (m)	2.45 (dd, 16.8, 7.7)	2.47 (dd, 16.9, 7.6)
9	2.96 (d, 7.4)	2.99 (d, 7.2)	2.96 (d, 7.3)	2.76 (d, 8.4)	2.92 (d, 7.6)	2.96 (d, 6.6)	2.99 (d, 7.0)	3.00 (d, 6.9)
11	5.54 (dd, 10.8, 7.4)	5.60 (dd, 10.7, 7.2)	5.58 (dd, 10.8, 7.3)	5.50 (dd, 11.9, 8.4)	5.45 (dd, 10.6, 7.6)	5.54 (dd, 10.4, 6.6)	5.62 (dd, 10.8, 7.0)	5.62 (dd, 10.8, 6.9)
12	5.71 (d, 10.8)	5.75 (d, 10.7)	5.74 (d, 10.8)	5.34 (d, 11.9)	5.66 (d, 10.6)	5.60 (d, 10.4)	5.52 (d, 10.8)	5.55 (d, 10.8)
15	3.84 (s)	3.87 (s)	3.84 (s)	4.77 (br t, 7.0)	3.88 (s)	3.91 (s)	3.93 (s)	3.93 (s)
16α	2.11 (dd, 14.1, 10.3)	2.12 (dd, 14.0, 10.2)	2.09 (m)	2.24 (dt, 14.0, 6.8)	2.17 (m)	1.82 (m)	1.80 (dd, 13.7, 11.2)	1.82 (m)
16β	2.28 (m)	2.30 (dt, 14.0, 7.3)	2.28 (m)	1.99 (dt, 14.0, 6.5)	2.29 (m)	2.40 (m)	2.35 (m)	2.42 (m)
17	2.79 (dd, 10.3, 7.3)	2.80 (dd, 10.2, 7.3)	2.79 (dd, 10.0, 7.4)	3.35 (dd, 13.7, 6.5)	2.94 (m)	2.87 (m)	2.97 (m)	3.04 (m)
18	0.97 (s, 3H)	1.01 (s, 3H)	0.99 (s, 3H)	0.91 (s, 3H)	0.93 (s, 3H)	0.94 (s, 3H)	1.13 (br s, 3H)	1.13 (s, 3H)
19	0.93 (s, 3H)	0.96 (s, 3H)	0.94 (s, 3H)	1.18 (s, 3H)	0.92 (s, 3H)	0.95 (s, 3H)	0.97 (s, 3H)	0.99 (s, 3H)
21				7.11 (br s)		5.73 (br s)	5.86 (s)	6.21 (br s)
22				6.16 (d, 1.0)	6.81 (s)	5.80 (s)	5.92 (s)	5.98 (s)
23				7.29 (t, 1.6)	5.95 (br s)			
28	0.97 (s, 3H)	1.00 (s, 3H)	0.98 (s, 3H)	1.07 (s, 3H)	0.92 (s, 3H)	0.99 (s, 3H)	0.98 (s, 3H)	1.00 (s, 3H)
29	1.07 (s, 3H)	1.09 (s, 3H)	1.08 (s, 3H)	0.95 (s, 3H)	1.04 (s, 3H)	1.01 (s, 3H)	1.09 (s, 3H)	1.10 (s, 3H)
30a	5.31 (s)	5.34 (s)	5.32 (s)	α 3.77 (d, 12.4)	5.31 (s)	5.34 (s)	5.36 (s)	5.37 (s)
30b	5.23 (s)	5.27 (s)	5.23 (s)	β 4.98 (d, 12.4)	5.21 (s)	5.27 (s)	5.30 (s)	5.30 (s)
7-OMe	3.66 (s, 3H)	3.68 (s, 3H)	3.67 (s, 3H)	3.79 (s, 3H)	3.62 (s, 3H)	3.66 (s, 3H)	3.68 (s, 3H)	3.69 (s, 3H)
11-OAc	1.91 (s, 3H)	1.92 (s, 3H)	1.91 (s, 3H)	2.02 (s, 3H)	1.50 (s, 3H)	1.66 (s, 3H)	1.93 (s, 3H)	1.94 (s, 3H)
2′	1.88 (s, 3H′)	2.38 (m)	2.13 (m)	1.64 (s, 3H)	3.33, 3.45 (d, 15.0)	3.42, 3.48 (d, 15.1)	2.32 (m)	2.13 (m)
3′		1.06 (d, 7.0, 3H)	1.28, 1.63 (m)				0.99 (d, 7.1, 3H)	1.29, 1.53 (m)
4′		1.04 (d, 7.0, 3H)	0.85 (t, 7.3, 3H)		7.18 (m)	7.16 (m)	1.02 (d, 7.1, 3H)	0.84 (t, 7.4, 3H)
5′			1.00 (d, 7.5, 3H)		7.23 (m)	7.30 (m)		1.01 (d, 7.0, 3H)
6′					7.20 (m)	7.25 (m)		
7′					7.23 (m)	7.30 (m)		
8′					7.18 (m)	7.16 (m)		

[a] Recorded in $CDCl_3$ at 400 MHz; δ in parts per million and J in hertz are in the parentheses.

Table 2. ^{13}C NMR spectroscopic data for compounds **1–8**[a]

Position	1	2	3	4	5	6	7	8
1	152.2	152.3	152.2	84.4	152.3	152.0	151.9	152.1
2	125.7	125.7	125.7	39.8	125.6	125.8	125.7	125.9
3	204.0	203.9	203.9	211.5	204.1	204.0	203.9	204.0
4	46.2	46.1	46.2	47.8	46.0	46.2	46.1	46.4
5	45.1	45.2	45.2	40.6	45.0	45.1	44.9	45.2
6	31.3	31.3	31.3	31.9	31.2	31.2	31.1	31.3
7	174.1	174.0	174.1	175.9	174.0	174.1	174.0	174.1
8	135.8	135.9	135.9	128.3	136.0	135.7	135.5	135.8
9	52.6	52.6	52.6	51.6	52.7	52.5	52.5	52.8
10	42.0	42.0	42.0	46.7	41.9	42.0	41.9	42.1
11	70.8	70.8	70.8	70.6	71.3	71.0/70.6	70.5	70.7
12	74.2	73.8	73.7	73.8	75.2	75.9/75.8	76.6	75.5
13	45.8	46.0	46.0	48.3	45.8	46.2	46.3	46.3
14	70.8	70.8	70.9	147.5	70.9	71.0	70.5	70.7
15	59.6	59.5	59.5	69.0	59.5	59.4/59.3	58.9	59.1
16	29.6	29.8	29.9	40.4	31.2	33.6/32.2	32.9	33.2
17	46.7	46.6	46.6	41.4	38.2	40.5/40.0	39.8	40.1
18	12.9	12.9	13.0	16.5	13.3	13.4/13.3	13.1	13.3
19	21.2	21.1	21.1	19.9	21.1	21.2	21.1	21.3
20	177.3	177.5	177.9	123.0	134.8	167.0/166.8	166.8	164.8
21				139.8	171.1	99.3	99.5	99.4[b]
22				110.7	147.6	120.3/118.9	118.5[b]	121.2[b]
23				142.2	96.2	170.4	170.2	170.4
28	22.9	22.9	22.9	23.5	22.8	22.9	22.8	22.9
29	22.6	22.6	22.7	24.8	22.5	22.6	22.5	22.7
30	121.5	121.4	121.4	68.5	121.2	122.1/122.0	122.2	121.9
7-OMe	52.1	52.0	52.1	52.3	52.0	52.1	52.1	52.2
11-OAc	169.7	169.6	169.7	170.3	169.7	169.6	169.5	169.6
	20.5	20.7	20.8	21.2	20.1	20.3	20.7	20.9
1′	170.0	175.6	175.2	170.8	170.3	171.7/170.3	176.0	176.6
2′	20.6	33.8	40.6	20.3	41.0	41.3	34.0	41.1
3′		18.2	25.6		133.2	132.4	18.1	25.7
4′		18.8	11.4		129.5	129.4	18.7	11.5
5′			16.1		128.4	128.8		15.8
6′					126.9	127.5		
7′					128.4	128.8		
8′					129.5	129.4		

[a] Recorded in $CDCl_3$ at 100 MHz; δ in parts per million.
[b] Carbon signal extremely broad or did not appear clearly; chemical shift values determined from HMBC and/or HMQC spectra.

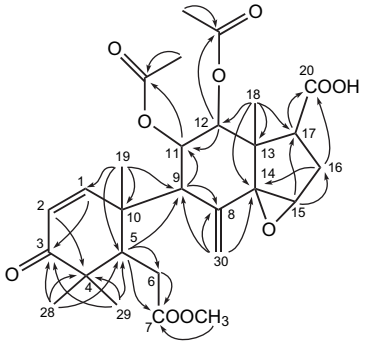


Figure 1. Selected HMBC correlations (H→C) of **1**.

highly resembled those of turrapubesin A (**13**),[5] with the main differences residing in the connection mode of rings A and C. In comparison with **13**, the C-3 resonance at δ_C 211.5 as assigned by HMBC correlations with H-1 at δ_H 3.63 (dd, J=3.8, 2.2 Hz), H-2α at 3.00 (dd, J=15.1, 3.8 Hz), and H-2β at 2.34 (dd, J=15.1, 2.2 Hz), was considerably downfield shifted. In addition, the methylene of C-30 (δ_C 68.5) was also severely downfield shifted as compared with that of **13** (δ_C 46.3).[5] The HMBC correlations from H-30α (δ_H 3.77, d, J=12.4 Hz) and H-30β (δ_H 4.98, d, J=12.4 Hz) to C-1 at δ_C 84.4 further indicated that a 1,30-

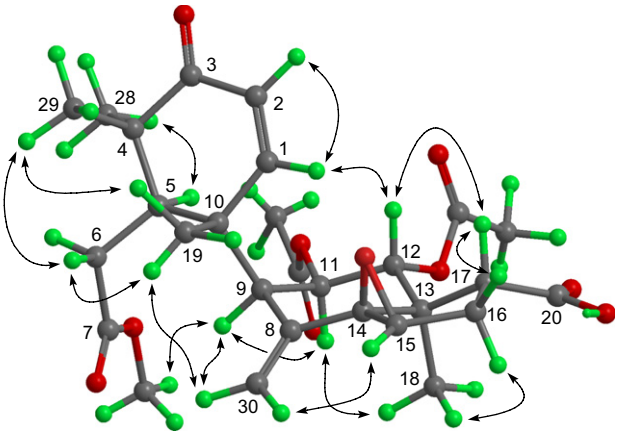


Figure 2. Key ROESY correlations (H↔H) of **1**.

oxygen bridge was present in **4** (Fig. 4). The planar structure of **4** was thus furnished.

The relative stereochemistry of **4** was deduced by the NOESY experiment (Fig. 5) together with interpretation of the ^{1}H NMR data. The coupling constants of $J_{9,11}$=8.4 Hz and $J_{11,12}$=11.9 Hz showed the typical pattern of a 11β,12α-substituted B-*seco* limonoids.[8] The NOESY correlations of

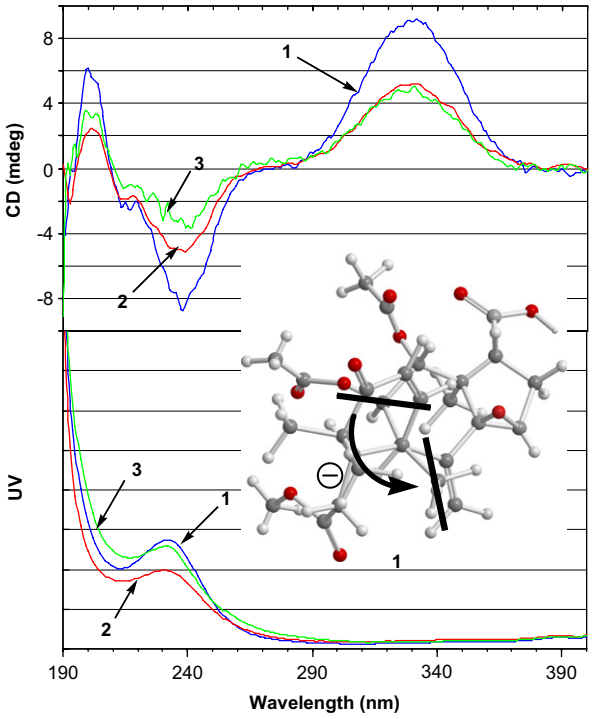


Figure 3. CD and UV spectra of **1**–**3**. Bold lines denote the electric transition dipole of the chromophores for **1**.

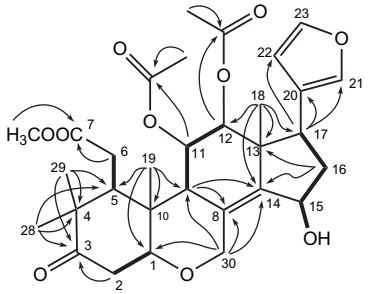


Figure 4. Selected ^{1}H–^{1}H COSY (bold lines) and HMBC correlations (H→C) of **4**.

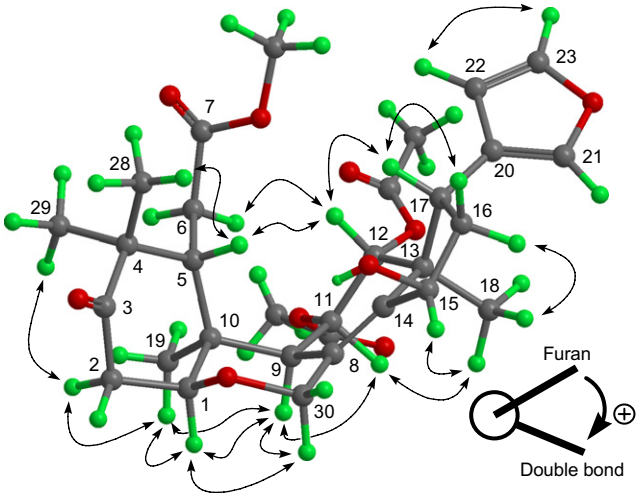
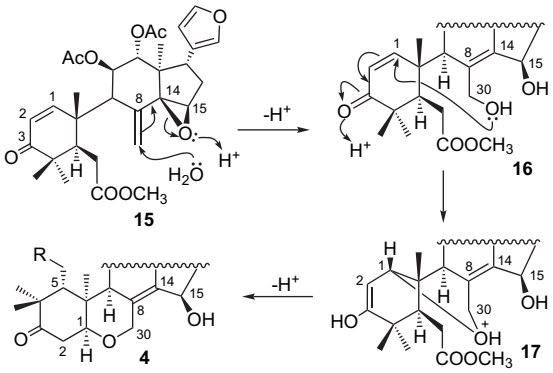


Figure 5. Key NOESY correlations (H↔H) of **4** and exciton coupling of furan and the $\Delta^{8(14)}$ double bond with positive chirality.

H-1/H-9, H-1/H-30α, and H-9/H-30α indicated that they were all in the axial position and α-oriented. The NOESY correlations of H-1/H_3-19, H-9/H_3-19, H-5/H-12, and H-6β/H-12 suggested that ring A and the newly generated tetrahydropyran ring were cis-fused between C-1 and C-10. Stereochemistry at other chiral centers was also determined by the NOESY spectrum (Fig. 5). The absolute configuration of turrapubesin C (**4**) was determined by the CD exciton chirality method.[9] The CD spectrum of **4** exhibited positive chirality resulting from the exciton coupling between the two different chromophores of the furan ring at 217 nm ($\Delta\varepsilon$ +12.66, $\pi\rightarrow\pi^*$ transition)[12] and the $\Delta^{8(14)}$ double bond at 200 nm ($\Delta\varepsilon$ −2.30, $\pi\rightarrow\pi^*$ transition),[11] indicating that the transition dipole moments of these two chromophores were oriented in a clockwise manner (Fig. 5). The absolute configuration of **4** was thus assigned as 1*S*, 5*R*, 9*S*, 10*S*, 11*R*, 12*R*, 13*S*, 15*R*, and 17*R*. The absolute configuration of **4** was consistent with that of turrapubesin A (**13**), whose absolute configuration was established by X-ray crystallography.[5]

Limonoids with a *seco*-ring B belonging to the toonafolin group[1c] usually possess a $\Delta^{8(30)}$ exocyclic double bond. However, both turrapubesin C (**4**) and the formerly reported turrapubesin A (**13**)[5] with a $\Delta^{8(14)}$ double bond are very rare for the toonafolin group, and **4** also possessed an unprecedented 1,30-oxygen bridge.[1c,3] A biosynthetic pathway was proposed for **4** starting from the precursor 11-*epi*-toonacilin (**15**)[8] (Scheme 1). Acid catalyzed opening of the 14,15-epoxy ring was followed by a cascade of hydroxylation and the $\Delta^{8(30)}$ double bond migration to form the intermediate **16**. A nucleophilic attack of 30-OH on C-1 of intermediate **16** would produce intermediate **17**, which was finally transformed to **4**.



Scheme 1. Plausible biogenetic pathway of **4**.

2.3. Turrapubesins D–G (5–8)

Turrapubesin D (**5**) was isolated as a white amorphous solid. The molecular formula was determined as $C_{37}H_{42}O_{11}$ by HREIMS at *m*/*z* 662.2720 $[M]^+$ (calcd 662.2727). The NMR data (Tables 1 and 2) exhibited characteristic signals for the tertiary methyls, the α,β-unsaturated ketone, the 8,30-double bond, the C-7 carbomethoxy, and the 14,15-epoxy group of a limonoid core that was the same as found in turrapubesic acid A (**1**). The proton signals at δ_H 6.81 (s, H-22) and 5.95 (br s, H-23) and carbon signals at δ_C 134.8 (C-20), 171.1 (C-21), 147.6 (C-22), and 96.2 (C-23) revealed

that the C-17 side chain was a 23-hydroxy-20(22)-ene-21,23-γ-lactone moiety, which was the same as that in 11-*epi*-23-hydroxytoonacilide (**11**)[3a] and was supported by HMBC correlations of H-22 to C-20, C-21, C-23, and C-17; and correlations of H-17 to C-20, C-21, and C-22. The NMR data indicated that the difference between **5** and **11** was the C-12 acyloxy group. The ^{1}H NMR spectrum of **5** showed two non-equivalent aliphatic protons at δ_H 3.33 (d, J=15 Hz, H-2′a) and 3.45 (d, J=15 Hz, H-2′b) and a group of aromatic protons at δ_H 7.18–7.23 (m, 5H), together with the carbon signals at δ_C 170.3 (C-1′), 41.0 (C-2′), 133.2 (C-3′), 129.5 (C-4′ and C-8′), 128.4 (C-5′ and C-7′), and 126.9 (C-6′) in the ^{13}C NMR, indicating the presence of a phenylacetyl group. The phenylacetoxyl group was attached at C-12 by the HMBC correlation between H-12 (5.66, d, J=10.6 Hz) and C-1′. The relative configuration of **5** was established by ROESY experiment (Supplementary data).

Turrapubesin E (**6**) was isolated as a white amorphous solid. The molecular formula was established as $C_{37}H_{42}O_{11}$ by HREIMS at *m/z* 662.2752 [M]$^+$ (calcd 662.2727), which was the same as that of **5**. The UV, IR, and NMR data (Tables 1 and 2) revealed that the structure of **6** was closely related with that of **5**, with the only difference being the C-17 side chain. A 21-hydroxy-20(22)-ene-21,23-γ-lactone moiety was assigned for the C-17 side chain on the basis of ^{1}H and ^{13}C NMR data (Tables 1 and 2), which was the same as that in 11-*epi*-21-hydroxytoonacilide (**12**).[3a] The relative configuration of **6** was consistent with that of **5** as determined by the ROESY experiment (Supplementary data). Compounds **5** and **6** are the first limonoids containing a phenylacetyl moiety found from the Meliaceae family.

Turrapubesin F (**7**), a white amorphous solid, was assigned the molecular formula of $C_{33}H_{42}O_{11}$ by HREIMS ([M]$^+$ at *m/z* 614.2718, calcd 614.2727). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **7** were similar to those of **6**, except that the phenylacetate functionality at C-12 in **6** was replaced by an isobutanoyloxy moiety in **7** [δ_H 2.32 (m), 0.99 (d, J=7.1 Hz, 3H), and 1.02 (d, J=7.1 Hz, 3H); δ_C 176.0, 34.0, 18.1, and 18.7]. Although the HMBC correlation from H-12 to C-1′ (δ_C 176.0) was not observed, the strong HMBC correlation from H-11 (δ_H 5.62, dd, J=10.8, 7.0 Hz) to the acetoxy carbonyl (δ_C 169.5) indicated that the acetate was attached to C-11, leaving the isobutanoyloxy moiety only assignable to C-12. This was supported by the downfield-shifted H-12 resonance at δ_H 5.52 (d, J=10.8 Hz) caused by acylation. The relative stereochemistry of **7** was tentatively assigned to be the same as that of **6** by their similar NMR data and CD spectra (Fig. 6).

Turrapubesin G (**8**), a white amorphous solid, showed a molecular ion peak at *m/z* 628.2908 in the HREIMS (calcd 628.2884), consistent with the molecular formula $C_{34}H_{44}O_{11}$. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **8** showed high similarity to those of **7**, except for the differences arising from the C-12 acyloxy groups. Further analysis of the 1D and 2D NMR of **8** indicated that the C-12 acyloxy moiety was a 2-methylbutanoyloxy group [δ_H 2.13 (m), 1.53 (m), 1.29 (m), 0.84 (t, J=7.4 Hz, 3H), and 1.00 (d, J=7.0 Hz, 3H); δ_C 176.6, 41.4, 25.7, 11.5, and 15.8], and its linkage to C-12 was determined by the HMBC correlation between H-12 and C-1′. The similar NMR data and CD spectra (Fig. 6) of **8** and **7** suggested that both compounds shared the same relative configuration.

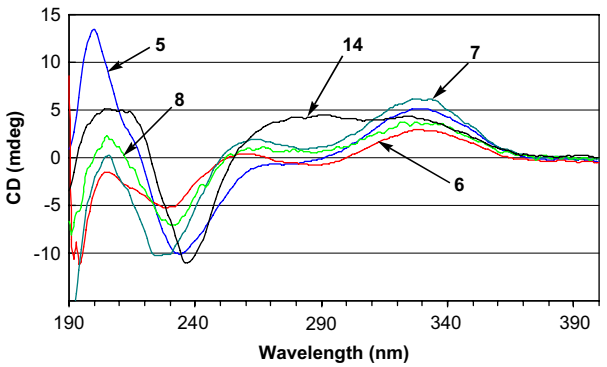


Figure 6. CD spectra of **5**–**8** and turrapubesin B (**14**).

The absolute configurations of turrapubesins D–G (**5**–**8**) were established by correlating their CD spectra to that of a model compound, turrapubesin B (**14**), a limonoid with established absolute configuration.[5] The CD curves (Fig. 6) of **5**–**8** matched well with that of **14**, indicating that their absolute configurations are identical to that of **14** with (5*R*,9*R*,10*S*,11*R*,12*R*,13*R*,14*S*,15*R*,17*R*)-configuration. Turrapubesins D−G (**5**–**8**) are a group of limonoids with a γ-hydroxybutenolide ring, which occurred as mixtures of the interconverting α- and β-hydroxy epimers of the hemiacetal carbon (C-21 or C-23) in solution.[3a,d,13] In consequence, some proton and carbon signals in the epimeric centers were observed in pairs, and even some other carbon signals near the epimeric centers were broadened or not resolved clearly.[3a,d,13] In these cases, the chemical shifts of some carbons were measured from the cross peaks in HMBC or HMQC spectra (Tables 1 and 2).

The cytotoxicity of **1**–**10** against the P-388 (murine leukemia) and A-549 (human lung adenocarcinoma) tumor cell lines was evaluated by using the MTT[14] and SRB[15] methods, respectively, with pseudolaric acid B[16] as the positive control (IC_{50} 0.74 μM against P-388 and 0.30 μM against A-549). Compounds **6** and **10** exhibited moderate activities against the P-388 cell line with the IC_{50} values of 16.0 and 12.3 μM, respectively. All the isolates were inactive against the A-549 cells.

3. Experimental section

3.1. General experimental procedures

Melting points were measured with an SGW X-4 melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. UV spectra were obtained on a Shimadzu UV-2550 spectrophotometer. CD spectra were obtained on a JASCO 810 spectrometer. IR spectra were obtained on a Perkin–Elmer 577 spectrometer in KBr discs. NMR spectra were recorded on a Bruker AM-400 spectrometer. EIMS (70 eV) was measured on a Finnigan MAT-95 mass spectrometer in *m/z* (rel %) and ESIMS was carried out on a Finnigan LC Q^{DECA} instrument. Semi-preparative HPLC was performed on a Waters 515

pump equipped with a Waters 2487 detector (254 nm) and a YMC-Pack ODS-A column (250×10 mm, S-5 μm, 12 nm). All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China). Silica gel (200–300 mesh), silica gel H60, Sephadex LH-20 (Amersham Biosciences), reversed-phase C_{18} silica gel (150–200 mesh, Merck), and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography. Pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd., Qingdao, People's Republic of China) were used for TLC.

3.2. Plant material

The twigs and leaves of *T. pubescens* Hellen were collected in August of 2003 from Hainan Province of the People's Republic of China. The plant was authenticated by Prof. Shi-Man Huang, Department of Biology, Hainan University of the People's Republic of China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, SIBS, Chinese Academy of Sciences (accession number: TP-2003-1Y).

3.3. Extraction and isolation

The air-dried powder of the plant (5 kg) was percolated with 95% EtOH, and the crude extract (600 g) was subsequently extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble fraction (211 g) was separated by silica gel column chromatography (CC) eluted with a gradient of petroleum ether/Me_2CO (10:1 to 0:1) to give six fractions (A–F). Fraction E (50 g) was then separated on an MCI-gel column (MeOH/H_2O, 50:50 to 90:10) to give six subfractions (E1–E6). Fraction E2 (4 g) was chromatographed over a silica gel column (petroleum ether/Me_2CO, 6:1 to 1:1) to afford four parts (E2a–E2d). E2c (0.7 g) was subjected to CC of reversed-phase C_{18} silica gel (MeOH/H_2O, 30:70 to 60:40) to give three parts (E2c1–E2c3). E2c1 (0.10 g) and E2c2 (0.21 g) were purified over silica gel columns (CH_2Cl_2/MeOH, 80:1) to afford **4** (30 mg) and **2** (24 mg), respectively. E2c3 (0.30 g) was separated by preparative HPLC (CH_3CN/H_2O, 60:40, 3 mL/min) to give **3** (20 mg) and **10** (30 mg). E2d (1.3 g) was further chromatographed over a silica gel column (CH_2Cl_2/MeOH, 100:1 to 30:1) to give four parts (E2d1–E2d4). E2d2 (0.20 g) was separated by preparative HPLC (CH_3CN/H_2O, 60:40, 3 mL/min) to give **7** (29 mg) and **8** (32 mg). E2d4 (0.33 g) was first separated on a column of RP-18 silica gel (MeOH/H_2O, 65:35), and the major components were then purified by Sephadex LH-20 (EtOH) to afford **1** (38 mg) and **9** (32 mg). Fraction E3 (7 g) was chromatographed through a column of reversed-phase C_{18} silica gel (MeOH/H_2O, 50:50 to 100:0) to afford six parts (E3a–E3f). E3b (0.42 g) was first subjected to CC of silica gel eluted with $CHCl_3$/MeOH (50:1), and then subjected to CC of silica gel eluted with petroleum ether/Me_2CO (2:1) to yield **5** (60 mg) and **6** (50 mg).

3.3.1. Turrapubesic acid A (1). White amorphous solid; $[\alpha]_D^{20}$ +87.0 (*c* 0.085, MeOH); UV (MeOH) λ_{max} (log ε) 233 (4.07) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (+2.36), 234 (−3.31), 328 (+3.48) nm; IR (KBr) ν_{max} 3433, 2956, 1738, 1680, 1437, 1369, 1279, 1236, 1047 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 532 $[M]^+$ (22), 472 (6), 454 (3), 412 (14), 397 (40), 379 (24), 337 (100), 312 (17), 209 (20), 149 (55), 121 (36); positive mode ESIMS *m/z* 555 $[M+Na]^+$ (100), 413 $[M+H-2(HOAc)]^+$ (6); negative mode ESIMS *m/z* 531 $[M-H]^-$ (55), 1085 $[2(M-H)+Na]^-$ (100); HREIMS *m/z* 532.2306 (calcd for $C_{28}H_{36}O_{10}$, 532.2308).

3.3.2. Turrapubesic acid B (2). Colorless needles; mp 201–202 °C; $[\alpha]_D^{20}$ +57.9 (*c* 0.190, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.68) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (+1.19), 239 (−2.48), 330 (+2.51) nm; IR (KBr) ν_{max} 3448, 2981, 1736, 1678, 1460, 1369, 1280, 1226, 1041, 929, 839 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; positive mode ESIMS *m/z* 583 $[M+Na]^+$ (100); negative mode ESIMS *m/z* 559 $[M-H]^-$ (100), 489 $[M-Me_2CHCO]^-$ (60), 617 $[M-H+Na^{35}Cl]^-$ (60); 619 $[M-H+Na^{37}Cl]^-$ (21); HRESIMS *m/z* 583.2539 (calcd for $C_{30}H_{40}O_{10}Na$, 583.2519).

3.3.3. Turrapubesic acid C (3). Colorless crystals; mp 156–157 °C; $[\alpha]_D^{20}$ +39.0 (*c* 0.110, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.01) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 199 (+1.26), 241 (−1.28), 330 (+1.76) nm; IR (KBr) ν_{max} 3435, 3174, 2983, 1739, 1707, 1682, 1458, 1367, 1282, 1225, 1041 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 574 $[M]^+$ (6), 472 (2), 412 (4), 397 (12), 379 (5), 337 (28), 209 (68), 149 (100), 121 (16); HREIMS *m/z* 574.2791 (calcd for $C_{31}H_{42}O_{10}$, 574.2778).

3.3.4. Turrapubesin C (4). White amorphous solid; $[\alpha]_D^{20}$ −215.0 (*c* 0.140, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.13) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (−2.30), 217 (+12.66), 284 (−1.16) nm; IR (KBr) ν_{max} 3437, 2952, 1747, 1718, 1637, 1460, 1437, 1373, 1246, 1028, 874, 793, 600 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 554 $[M-H_2O]^+$ (90), 494 (45), 452 (16), 434 (92), 286 (20), 226 (100), 210 (36), 149 (32), 121 (33); positive mode ESIMS *m/z* 595 $[M+Na]^+$ (100), 577 $[M+Na-H_2O]^+$ (13); HRESIMS *m/z* 595.2512 (calcd for $C_{31}H_{40}O_{10}Na$, 595.2519).

3.3.5. Turrapubesin D (5). White amorphous solid; $[\alpha]_D^{20}$ +70.3 (*c* 0.165, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.99) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (+5.42), 234 (−4.04), 328 (+2.07) nm; IR (KBr) ν_{max} 3435, 2956, 1755, 1676, 1369, 1230, 1041, 929, 708 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 662 $[M]^+$ (10), 526 (2), 466 (13), 391 (28), 297 (36), 239 (25), 210 (22), 149 (28), 91 (100); HREIMS *m/z* 662.2720 (calcd for $C_{37}H_{42}O_{11}$, 662.2727).

3.3.6. Turrapubesin E (6). White amorphous solid; $[\alpha]_D^{20}$ +53.9 (*c* 0.180, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.08) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 205 (−0.61), 228 (−2.10), 328 (+1.18) nm; IR (KBr) ν_{max} 3429, 2956, 1751, 1678, 1456, 1369, 1228, 1140, 1041, 706 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 662 $[M]^+$ (2), 526 (2), 466 (20), 391 (61), 297 (56), 239 (44), 210 (58), 149 (100), 91 (85); HREIMS *m/z* 662.2752 (calcd for $C_{37}H_{42}O_{11}$, 662.2727).

3.3.7. Turrapubesin F (7). White amorphous solid; $[\alpha]_D^{20}$ +40.9 (*c* 0.110, MeOH); UV (MeOH) λ_{max} (log ε) 228

(3.83) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 206 (+0.13), 225 (−5.44), 328 (+3.30) nm; IR (KBr) ν_{max} 3431, 2958, 1753, 1680, 1369, 1226, 1144, 1039, 929 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 614 $[M]^+$ (4), 466 (4), 391 (13), 297 (9), 209 (48), 149 (100), 71 (35); positive mode ESIMS *m/z* 637 $[M+Na]^+$ (100), 1251 $[2M+Na]^+$ (4); negative mode ESIMS *m/z* 613 $[M-H]^-$ (100), 1249 $[2(M-H)+Na]^-$ (11); HREIMS *m/z* 614.2718 (calcd for $C_{33}H_{42}O_{11}$, 614.2727).

3.3.8. Turrapubesin G (8). White amorphous solid; $[\alpha]_D^{20}$ +29.0 (*c* 0.105, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.84) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 205 (+0.91), 231 (−2.71), 321 (+1.41) nm; IR (KBr) ν_{max} 3429, 2925, 1753, 1680, 1460, 1369, 1226, 1142, 1039 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 628 $[M]^+$ (2), 466 (5), 391 (17), 297 (14), 239 (15), 209 (43), 149 (98), 57 (100); positive mode ESIMS *m/z* 651 $[M+Na]^+$ (100), 1279 $[2M+Na]^+$ (24); negative mode ESIMS *m/z* 627 $[M-H]^-$ (100), 1255 $[2M-H]^-$ (7); HREIMS *m/z* 628.2908 (calcd for $C_{34}H_{44}O_{11}$, 628.2884).

Acknowledgements

Financial support from the Key Project of National Natural Science Foundation (Grant No. 30630072), and the foundation from the Ministry of Science and Technology (Grant No. 2002CB512807) of the People's Republic of China is gratefully acknowledged. We thank Prof. S.-M. Huang for the collection and identification of the plant material.

Supplementary data

The MS, IR, and 1D and 2D NMR spectra of **1**–**8** are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.05.107.

References and notes

1. (a) Taylor, D. A. H. *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer: New York, NY, 1984; Vol. 45, pp 1–102; (b) Champagne, D. E.; Koul, O.; Isman, M. B.; Scudder, G. G. E.; Towers, G. H. N. *Phytochemistry* **1992**, *31*, 377–394; (c) Mulholland, D. A.; Parel, B.; Coombes, P. H. *Curr. Org. Chem.* **2000**, *4*, 1011–1054; (d) Roy, A.; Saraf, S. *Biol. Pharm. Bull.* **2006**, *29*, 191–201.
2. (a) Chen, S. K.; Chen, B. Y.; Li, H. *Flora of China (Zhongguo Zhiwu Zhi)*; Science: Beijing, 1997; Vol. 43, Part 3, p 50; (b) The Editorial Committee of the Administration Bureau of Traditional Chinese Medicine. *Chinese Materia Medica (Zhonghua Benchao)*; Shanghai Science and Technology: Shanghai, 1999; Vol. 5, p 49.
3. (a) Cheplogoi, P. K.; Mulholland, D. A. *Phytochemistry* **2003**, *62*, 1173–1178; (b) Ndung'u, M.; Hassanali, A.; Hooper, A. M.; Chhabra, S.; Miller, T. A.; Paul, R. L.; Torto, B. *Phytochemistry* **2003**, *64*, 817–823; (c) Cheplogoi, P. K.; Mulholland, D. A. *Biochem. Syst. Ecol.* **2003**, *31*, 799–803; (d) McFarland, K.; Mulholland, D. A.; Fraser, L. A. *Phytochemistry* **2004**, *65*, 2031–2037; (e) 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.
4. Wang, X. N.; Fan, C. Q.; Yue, J. M. *Steroids* **2006**, *71*, 720–724.
5. 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.
6. Rodriguez, B. *Magn. Reson. Chem.* **2003**, *41*, 206–212.
7. Qi, S. H.; Chen, L.; Wu, D. G.; Ma, Y. B.; Luo, X. D. *Tetrahedron* **2003**, *59*, 4193–4199.
8. Mulholland, D. A.; Monkhe, T. V.; Coombes, P. H.; Rajab, M. S. *Phytochemistry* **1998**, *49*, 2585–2590.
9. Berova, N.; Nakanishi, K. *Circular Dichroism: Principles and Applications*, 2nd ed.; Berova, N., Nakanishi, K., Woody, R. W., Eds.; Wiley-VCH: New York, NY, 2000; pp 337–382.
10. Koreeda, M.; Weiss, G.; Nakanishi, K. *J. Am. Chem. Soc.* **1973**, *95*, 239–240.
11. Harada, N.; Iwabuchi, J.; Yokota, Y.; Uda, H.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 5590–5591.
12. (a) Zhou, J. B.; Tadera, K.; Minami, Y.; Yagi, F.; Kurawaki, J.; Takezaki, K.; Nakatani, M. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 496–500; (b) Abdelgaleil, S. A. M.; Okamura, H.; Iwagawa, T.; Sato, A.; Miyahara, I.; Doe, M.; Nakatani, M. *Tetrahedron* **2001**, *57*, 119–126.
13. (a) Garcez, F. R.; Garcez, W. S.; Tsutsumi, M. T.; Roque, N. F. *Phytochemistry* **1997**, *45*, 141–148; (b) Shirota, O.; Nagamatsu, K.; Sekita, S. *J. Nat. Prod.* **2006**, *69*, 1782–1786.
14. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.
15. Skehan, P. A.; Storeng, R.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
16. Pan, D. J.; Li, Z. L.; Hu, C. Q.; Chen, K.; Chang, J. J.; Lee, K. H. *Planta Med.* **1990**, *56*, 383–385.