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Cytotoxic limonoids from *Trichilia americana* leaves

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ABSTRACT

Ten cedrelone limonoids were isolated from the leaves of *Trichilia americana*. These compounds include americanolides A–D (**1**–**4**), 1,2-dihydrodeacetylhirtin (**5**), 1α-hydroxy-1,2-dihydrodeacetylhirtin (**6**), 1α-hydroxy-1,2-dihydrohirtin (**7**), 1α-methoxy-1,2-dihydrodeacetylhirtin (**8**), 11β-hydroxy-12α-propanoyloxycedrelone (**9**), and 1α,11β-dihydroxy-1,2-dihydrocedrelone (**10**), as well as two previously reported compounds, deacetylhirtin (**11**) and hirtin (**12**). Their structures were characterized on the basis of spectroscopic studies, and the assignment of the absolute configuration of americanolide A (**1**) was supported by single-crystal X-ray diffraction studies. The cytotoxic activities of all isolated compounds were also evaluated against five human tumour cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) using an MTS assay. Compounds **11** and **12** showed significant cytotoxicity with IC_{50} values ranging from 0.1 to 0.5 μM, and compounds **5**, **6**, **7**, **8**, **9**, and **10** exhibited potent or selective cytotoxic activity with IC_{50} values ranging from 1.0 to 39.6 μM.

1. Introduction

Trichilia P. Br. (Meliaceae) is a genus with approximately 419 species that are primarily distributed throughout the tropical areas of America, Africa, India, Indo-China, and the Malaya Peninsula of Asia (Chen et al., 1997; Garima, 2011). Many species in this genus have been widely used as folk medicines, such as two of the three Chinese species, namely *Trichilia connaroides* and *Trichilia sinensis*, which are often used to treat arthritis, pharyngitis, tonsillitis, chronic osteomyelitis, scabies, eczema, and abdominal pain (Garima, 2011; Xu et al., 2013). Previous phytochemical studies have shown that this genus afforded a series of protolimonoids and limonoids with a variety of therapeutic potential effects such as antifeedant, antioxidant, cytotoxic, and anti-inflammatory activities (Garima, 2011; Tan and Luo, 2011; Zhang et al., 2011; Piaz et al., 2012; Vieira et al., 2013; Terra et al., 2013; Wang et al., 2013a,,b; Xu et al., 2013).

Trichilia americana is native to America (Wheeler and Isman, 2000, 2001), but it was also introduced to the Xishuangbannan Tropical Botanical Garden (XTBG), Chinese Academy of Sciences in the 1990s (Li et al., 1996). The methanol extract of *T. americana* showed antifeedant and cytotoxic effects against the Asian armyworm (*Spodoptera litura*) (Wheeler and Isman, 2000, 2001). However, the chemical constituents in this plant species were not reported. As a continuation of research about biologically active protolimonoids and limonoids in the Meliaceae family (Tang et al., 2012; Ji et al., 2014a,b), ten new (**1**–**10**) and two previously reported (**11**–**12**) cedrelone limonoids were isolated from the leaves of *T. americana*. Their structures were established on the basis of extensive spectroscopic analysis and by comparisons with data from the literature. The absolute configuration of **1** was also confirmed by single crystal X-ray diffraction. All isolated compounds were assessed for their cytotoxic activities against five human tumour cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480). Herein described are the isolation, structural elucidation, and biological evaluation of these isolated compounds.

2. Results and discussion

The leaves of *T. americana* were subjected to a general extraction with EtOH. The solvent was removed, and the ethanol extract

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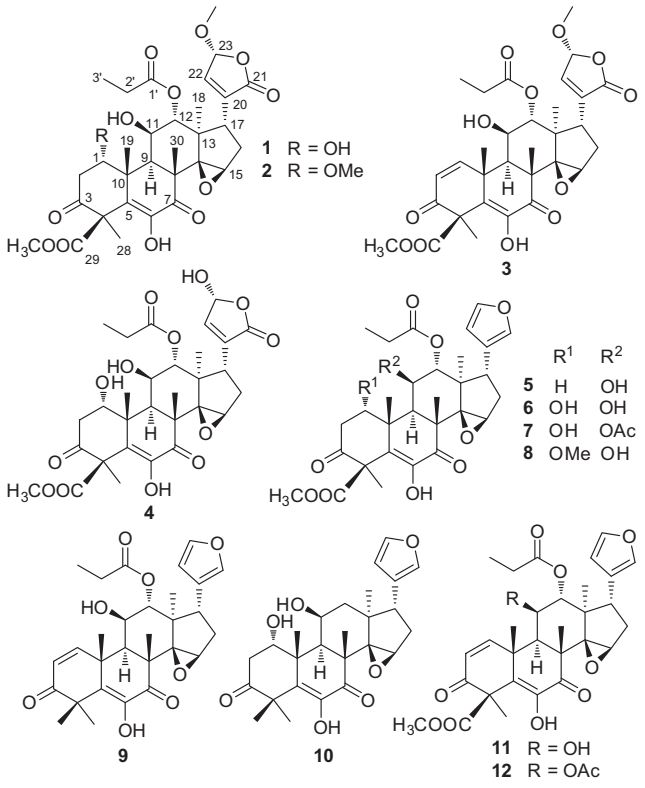

Fig. 1. Structures of isolated compounds **1**–**12**.

was suspended in water and then partitioned with EtOAc. The EtOAc fraction solution was purified by column chromatography, and ten new (**1**–**10**) and two known (**11**–**12**) compounds were obtained as shown in Fig. 1.

Compound **1** was isolated in the form of colourless crystals. Its molecular formula of $C_{31}H_{38}O_{13}$ was determined by HR-ESI(+)MS at *m*/*z* 641.2200 $[M+Na]^+$ (calcd 641.2205) by incorporating 13 degrees of unsaturation. Its IR spectrum indicated the presence of hydroxyl (3433 cm^{-1}) and carbonyl (1735 cm^{-1}) groups. The 1D NMR spectra (Tables 1 and 2) of **1** and the ^1H–^{13}C HSQC spectrum showed that this compound contains an ester (δ_H 3.69 and δ_C 171.3), a ketone (δ_C 208.2), an epoxy (δ_H 3.92 and δ_C 55.2, 68.3), and an α-hydroxy-α,β-unsaturated ketone (δ_C 133.2, 142.5, and 197.3) groups. The typical resonances for a propanoyl side-chain were identified at δ_H 1.06 (t, *J* = 7.5), 2.24 (q, *J* = 7.8) and δ_C 8.8, 27.8, 174.9, as well as a methoxy-substituted α,β-unsaturated-γ-lactone ring at δ_H 3.50, 5.71, 6.76 and δ_C 56.9, 102.4, 136.9, 145.1, 170.9 (Ji et al., 2014a). The observed NMR spectroscopic signals above established that the compound still required five additional degrees of unsaturation, suggesting hexacyclic rings. In addition, four tertiary methyl groups (δ_H 0.83, 1.34, 1.38, 1.82 and 14.8, 17.9, 19.7, 22.6) were also present in compound **1** according to an analysis of ^1H–^{13}C HSQC data. These observations suggested that **1** was possibly a cedrelone limonoid (Simmonds et al., 2001).

A comparison of the ^1H and ^{13}C NMR spectroscopic data of **1** (Tables 1 and 2) with those of deacetylhirtin (**11**) (Simmonds et al., 2001) implied that they shared a similar skeleton, with a noticeable difference being in the presence of a methoxy-substituted α,β-unsaturated-γ-lactone ring at the C-17 position as observed at δ_H 3.50, 5.71, 6.76 and δ_C 56.9, 102.4, 136.9, 145.1, 170.9, instead of the furan ring (Simmonds et al., 2001; Luo et al., 2000). The results were further confirmed by a ^1H–^{13}C HMBC correlation between OCH_3 (δ_H 3.50) and C-23 (δ_C 56.9) and a ^1H–^1H COSY correlation from H-22 to H-23 (Fig. 2A). The NMR signal of CH-1 at δ_H 4.19 (d, *J* = 5.9) and δ_C 70.3 together with the ^1H–^1H COSY correlation between H-1 and H-2 and the ^1H–^{13}C HMBC cross-peak from methyl H-19 to C-1 (Fig. 2A) indicated that CH-1 was a hydroxyl-substituted oxymethine. The propanoyl group was located at C-12 according to the HMBC correlation between H-12 and C-1′ (Fig. 2A). The key ^1H–^{13}C HMBC correlations from proton resonances of H-18 and H-30 to C-14, and the ones from H-15 to C-16 inferred that the epoxy group should be located between C-14 and C-15. Thus, the structure of compound **1** was established.

Table 1
^1H NMR spectroscopic data for compounds **1**–**5**.

No.	**1**[a] (mult., *J* in Hz)	**2**[b] (mult., *J* in Hz)	**3**[b] (mult., *J* in Hz)	**4**[b] (mult., *J* in Hz)	**5**[c] (mult., *J* in Hz)
1α			7.12 (d, 10.2)		1.98 (m)
1β	4.19 (d, 5.9)	3.73 (d, 6.3)		4.16 (d, 6.0)	2.26 (m)
2α	2.67 (d, 19.4)	2.74 (m, 2H)	6.14 (d, 10.1)	2.69 (d, 19.3)	2.85 (dd, 19.0, 8.7)
2β	2.90 (m)			2.90 (dd, 19.3, 6.1)	2.70 (dt, 18.9, 9.3)
9	3.26 (s)	3.29 (s)	2.68 (s)	3.36 (s)	2.90 (s)
11	4.06 (s)	4.10 (s)	4.33 (s)	4.13 (s)	4.52 (s)
12	4.89 (s)	4.98 (s)	5.05 (s)	4.96 (s)	5.71 (s)
15	3.92 (s)	3.89 (s)	3.91 (s)	3.89 (s)	4.06 (s)
16α	1.96 (m)	2.11 (dd, 13.4, 11.6)	2.11 (m)	2.12 (m)	2.06 (m)
16β	2.20 (m)	2.18 (m)	2.19 (m)	2.18 (dd, 13.6, 6.7)	2.20 (dd, 13.6, 6.7)
17	2.77 (dd, 10.8, 6.6)	2.77 (m)	2.77 (dd, 10.9, 6.8)	2.74 (m)	3.10 (dd, 10.8, 6.6)
18	0.83 (s, 3H)	0.87 (s, 3H)	0.83 (s, 3H)	0.88 (s, 3H)	1.06 (s, 3H)
19	1.38 (s, 3H)	1.39 (s, 3H)	1.63 (s, 3H)	1.37 (s, 3H)	1.80 (s, 3H)
21					7.34 (s)
22	6.76 (s)	7.08 (s)	7.08 (br s)	7.04 (s)	6.23 (br s)
23	5.71 (s)	5.86 (s)	5.85 (br s)	6.10 (s)	7.52 (br s)
28	1.82 (s, 3H)	1.74 (s, 3H)	1.76 (s, 3H)	1.82 (s, 3H)	2.04 (s, 3H)
30	1.34 (s, 3H)	1.36 (s, 3H)	1.42 (s, 3H)	1.35 (s, 3H)	1.84 (s, 3H)
2′	2.24 (q, 7.8, 2H)	2.27 (q, 7.5, 2H)	2.29 (m, 2H)	2.26 (m, 2H)	2.35 (m, 2H)
3′	1.06 (t, 7.5, 3H)	1.09 (t, 7.6, 3H)	1.11 (t, 7.6, 3H)	1.07 (t, 6.9, 3H)	1.05 (t, 7.5, 3H)
OMe-1		3.31 (s, 3H)			
OMe-23	3.50 (s, 3H)	3.53 (s, 3H)	3.52 (s, 3H)		
OMe-29	3.69 (s, 3H)	3.67 (s, 3H)	3.73 (s, 3H)	3.67 (s, 3H)	3.79 (s, 3H)

[a] Measured in $CDCl_3$ at 600 MHz.
[b] Measured in CD_3OD at 600 MHz.
[c] Measured in C_5D_5N at 400 MHz.

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**10**.

No.	1[a]	2[b]	3[b]	4[b]	5[c]	6[c]	7[d]	8[a]	9[a]	10[a]
1	70.3	81.7	154.2	71.3	35.3	71.1	71.1	80.5	151.0	70.9
2	43.4	38.2	127.1	44.5	33.6	44.9	44.6	37.4	127.8	43.9
3	208.2	210.0	198.8	210.7	209.9	209.8	209.5	207.1	203.5	213.2
4	59.6	60.9	60.9	61.1	60.7	61.0	61.1	59.5	48.6	47.9
5	133.2	133.7	130.4	134.3	136.7	134.4	134.6	132.5	134.7	135.4
6	142.5	144.8	144.5	144.8	143.56	145.9	146.0	142.2	140.9	143.0
7	197.3	198.3	197.7	198.5	198.6	198.9	198.0	196.9	197.3	197.7
8	45.4	47.1	47.8	47.0	47.6	47.3	47.1	45.4	45.5	45.3
9	38.8	39.7	45.1	39.8	47.4	39.7	39.1	38.7	43.4	39.8
10	43.3	44.5	41.4	44.3	38.9	44.3	44.0	43.6	40.5	44.4
11	71.9	72.8	72.5	72.5	72.9	73.2	74.2	73.7	73.3	67.1
12	84.5	85.4	84.5	85.3	82.7	84.0	81.0	84.2	82.8	46.3
13	44.7	46.0	45.9	45.9	45.5	45.6	46.0	44.7	44.9	41.3
14	68.3	69.5	69.1	69.6	68.8	69.2	69.1	68.6	68.4	70.0
15	55.2	56.2	56.0	56.3	55.4	55.6	56.2	56.7	56.6	59.2
16	30.9	31.7	31.7	31.7	32.1	32.3	33.0	31.7	31.6	31.4
17	41.6	42.9	42.9	42.9	42.6	42.7	42.6	42.5	42.3	42.6
18	14.8	15.4	15.7	15.4	16.4	16.0	15.6	15.0	15.5	22.1
19	17.9	18.2	27.7	18.3	18.6	18.7	17.6	17.7	26.2	16.8
20	136.9	137.4	137.3	136.6	123.6	123.8	123.5	122.3	122.1	122.9
21	170.9	173.0	172.9	173.5	140.8	140.8	141.4	140.2	140.2	139.6
22	145.1	147.7	147.7	149.3	111.9	112.0	112.4	111.3	111.1	110.7
23	102.4	104.3	104.3	99.0	143.61	143.5	143.5	142.9	143.0	143.3
28	19.7	20.0	23.5	20.2	20.6	20.7	20.7	19.7	27.0	24.4
29	171.3	173.1	172.1	173.3	172.2	172.5	172.5	171.0	21.4	21.1
30	22.6	22.9	22.9	23.0	23.5	23.5	23.2	22.8	22.8	23.0
1'	174.9	175.1	175.1	175.6	173.9	174.1	173.1	174.2	174.0	
2'	27.8	28.7	28.6	28.6	28.5	28.3	28.1	27.9	28.0	
3'	8.8	9.2	9.3	9.2	9.8	9.4	9.4	9.1	9.2	
OMe-1		57.2						56.5		
OAc-11							170.3 21.6			
OMe-23	56.9	57.1	57.1							
OMe-29	52.9	53.0	53.1	53.0	52.8	52.8	53	53.0		

[a] Measured in $CDCl_3$ at 150 MHz.
[b] Measured in CD_3OD at 150 MHz.
[c] Measured in C_5D_5N at 100 MHz.
[d] Measured in C_5D_5N at 150 MHz.

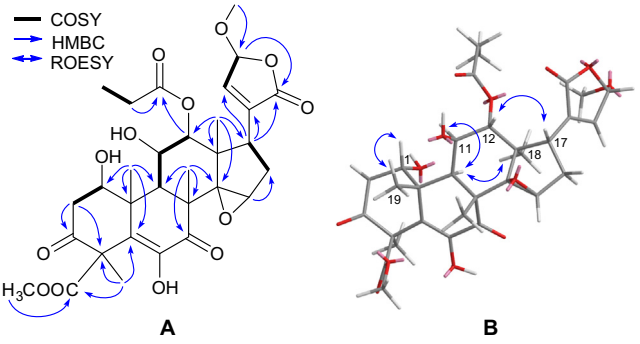

Fig. 2. Selected COSY (bold bond), HMBC (A) and ROESY (B) correlations of **1**.

The relative configuration of **1** was primarily derived by a ROESY experiment (Fig. 2B). The NOE interactions of H-19/H-1, H-12/H-17, and H-17/H-16β were observed, indicating that they were co-facial with a β-orientation, whereas the strong correlations of H-9/H-11 and H-9/H-18 established that H-9, H-11, and H-18 were α-configured (Fig. 2B). Although there was an absence of a ROESY correlation with other protons in CH-23, a single-crystal X-ray diffraction study (Fig. 3) further confirmed the above speculation. The refined Hooft parameter value was 0.22(19) for 4386 Bijvoet pairs with a probability of 1.000, and the previous results allowed for the assignment of the absolute configuration of **1** as 1*S*, 4*R*, 8*R*, 9*R*, 10*R*, 11*R*, 12*R*, 13*R*, 14*R*, 15*R*, 17*R*, and 23*S* (Hooft et al., 2008). This compound was named americanolide A.

Compound **2** was obtained in the form of colourless crystals. Its HR-ESI(+)MS at *m*/*z* 655.2365 $[M+Na]^+$ (calcd 655.2361) was consistent with a molecular formula of $C_{32}H_{40}O_{13}$. A detailed analysis of the NMR spectroscopic data (Tables 1 and 2) showed that it was very similar to those of **1**, with the difference being the presence of an additional methoxy group. The downfield shift of signal for the oxymethine (C-1) from δ_C 70.3 to 81.7 and the ^{1}H–^{13}C HMBC correlations from OCH_3 (δ_H 3.31) to C-1 and H-1 (δ_H 3.73, d, J = 6.3) to OCH_3-1 (δ_C 57.2) indicated that this methoxy group should be located at C-1. The ^{1}H–^{1}H ROESY correlation between H-1 and methyl H-19 suggested that the methoxy group was α-configured (see Supporting information). Compound **2** was therefore established as americanolide B.

Compound **3**, which was in the form of colourless crystals, possessed the molecular formula $C_{31}H_{36}O_{12}$ as determined by HR-ESI(+)MS at *m*/*z* 623.2103 $[M+Na]^+$ (calcd 623.2099). The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **3** resembled those of compound **1** except for the replacement of signals for an oxymethine that was observed at CH-1 with δ_H 4.19 (d, J = 5.9) and δ_C 70.3, and a methylene at CH_2-2 with δ_H 2.67 (d, J = 19.4), 2.90 (m) and δ_C 43.4 groups in **1** by those for the $\Delta^{1,2}$ double bond at δ_H 6.14 (d, J = 10.1), 7.12 (d, J = 10.2) and δ_C 127.1, 154.2. These observations suggested that **3** could be a $\Delta^{1,2}$ double bond derivative of **1**. A further ^{1}H–^{1}H COSY correlation between H-1 at δ_H 6.14 (d, J = 10.1) and H-2 at δ_H 7.12 (d, J = 10.2), and the ^{1}H–^{13}C HMBC correlations from the methyl protons H-19 (δ_H 1.38) to C-1 and from H-1 to C-3 (δ_C 208.2) (see Supporting information) confirmed that the structure that was elucidated for **3** was americanolide C.

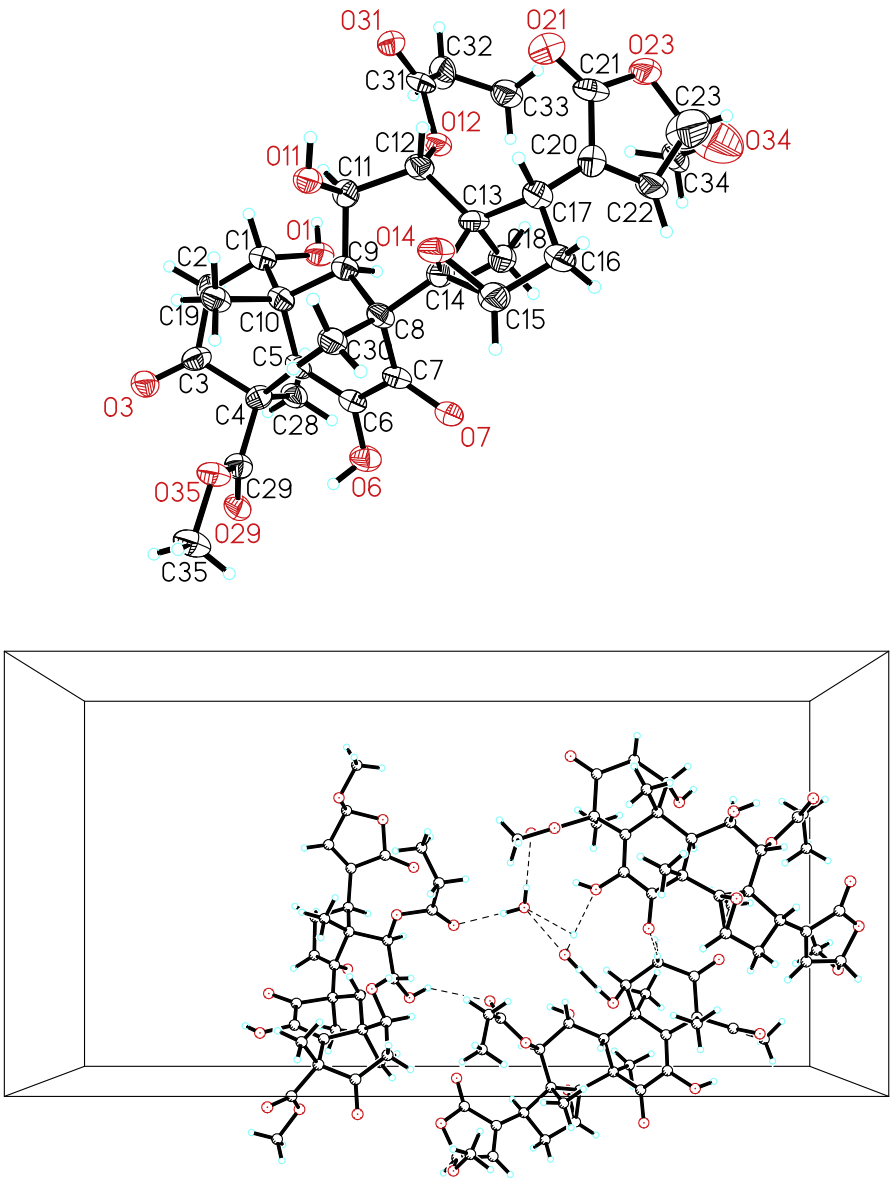

Fig. 3. Single-crystal X-ray structure of **1** and the hydrogen bonding network between three molecules of **1** and two water molecules (hydrogen-bonds are shown as dashed lines).

The molecular formula of compound **4** ($C_{30}H_{36}O_{13}$) was established by HR-ESI(+)MS at *m*/*z* 627.2058 $[M+Na]^+$ (calcd 627.2048), for 14 mass units less than that of **1**. The NMR spectroscopic data of **4** differed from those of **1** (Tables 1 and 2) only in the absence of a signal for the methoxy (OCH_3-23; δ_H 3.50; δ_C 56.9) group in α,β-unsaturated-γ-lactone ring at C-17 and the presence of a hydroxyl group. The upfield shift in the carbon signal for the dioxymethine (C-23) from δ_C 102.4 to 99.0 also confirmed this assignment, and this compound has been named americanolide D.

Compound **5** was obtained as colourless crystals and showed a molecular formula of $C_{30}H_{36}O_{10}$ as established by the quasi-molecular ion peak at *m*/*z* 557.2400 $[M+H]^+$ in the positive-ion mode via HR-ESI(+)MS. An analysis of the 1D NMR data combined with the 2D NMR spectra of **5** exhibited signals for a β-substituted furan ring, an 5,6-enol-7-one ring B, four angular methyls, and a methyl-esterified C-29 appendage, suggesting that **5** was also a cedrelone-class limonoid (Tan and Luo, 2011). A comparison of the NMR data for **5** with those of deacetylhirtin (**11**) (Simmonds et al., 2001) indicated that their structures were closely related, with a major difference in the replacement of signals for the $\Delta^{1,2}$ double bond observed at δ_H 6.13 (d, J = 10.3), 6.91 (d, J = 10.3) and δ_C 126.5, 151.4 in deacetylhirtin (**11**) by those with two additional sp^3 methylene groups observed at δ_H 1.98 (m), 2.26 (m), 2.70 (dt, J = 18.9, 8.7), 2.85 (dd, J = 19.0, 8.7) and δ_C 33.6, 35.3 (Tables 1 and 2). The ^1H–^{13}C HMBC cross-peaks of H-19/C-1, H-1/C-3, and H-2/C-4 along with a ^1H-^1H COSY correlation from H-1 to H-2 proton signals known as **5** was a 1,2-dihydro derivative of deacetylhirtin (**11**) and was named 1,2-dihydrodeacetylhirtin.

Compound **6** was obtained as colourless crystals. The HR-ESI(+)MS ion at *m*/*z* 573.2342 $[M+H]^+$ (calcd 573.2330) provided the molecular formula $C_{30}H_{36}O_{11}$ to **6**. The NMR spectroscopic data (Tables 2 and 3) for **6** showed high similarities to those of **5**, apart from the presence of one additional hydroxy group. In addition, the carbon signal of CH-1 in the ^{13}C NMR data was downfield shifted from δ_C 35.3 to 71.1, indicating that the hydroxyl group should be located at C-1. The ^1H–^1H ROESY correlation between H-1 and methyl H-19 suggested that the hydroxyl group was α-configured (see Supporting information). These observations indicated that

Table 3
^{1}H NMR spectroscopic data for compounds **6**–**10**.

No.	**6**[a] (mult., *J* in Hz)	**7**[b] (mult., *J* in Hz)	**8**[c] (mult., *J* in Hz)	**9**[c] (mult., *J* in Hz)	10[c] (mult., *J* in Hz)
1α				6.82 (d, 10.0)	
1β	4.60 (m)	4.75 (m)	3.68 (d, 5.7)		4.27 (t, 5.4)
2α	3.07 (d, 19.2)	3.10 (d, 19.1)	2.83 (d, 19.1)	6.13 (d, 9.9)	2.70 (d, 19.2)
2β	3.20 (dd, 19.0, 5.7)	3.19 (dd, 19.0, 5.7)	2.74 (dd, 19.1, 5.8)		2.99 (dd, 19.2, 6.5)
9	4.06 (s)	4.35 (s)	3.37 (s)	2.69 (s)	3.07 (s)
11	4.67 (d, 3.1)	5.87 (s)	3.97 (s)	4.20 (d, 5.4)	4.44 (m)
12	5.71 (s)	5.67 (s)	5.02 (s)	5.1 (s)	2.04 (m, 2H)
15	4.11 (s)	4.14 (s)	3.97 (s)	3.98 (s)	4.02 (s)
16α	2.02 (m)	2.08, (m)	2.02 (dd, 13.1 11.8)	2.02 (dd, 13.6, 11.2)	2.00 (m)
16β	2.20 (dd, 13.5, 6.6)	2.23 (dd, 13.6, 6.6)	2.33 (dd, 13.8, 6.6)	2.34 (dd, 13.9, 6.6)	2.36 (dd, 13.7, 6.5)
17	3.12 (m)	3.16 (m)	2.90 (dd, 11.1, 6.6)	2.93 (dd, 11.0, 6.6)	2.81 (dd, 10.8, 6.6)
18	1.18 (s, 3H)	1.21 (s, 3H)	0.83 (s, 3H)	0.78 (s, 3H)	0.72 (s, 3H)
19	1.88 (s, 3H)	1.50 (s, 3H)	1.43 (s, 3H)	1.54 (s, 3H)	1.31 (s, 3H)
21	7.30 (s)	7.37 (s)	7.13 (s)	7.14 (s)	7.16 (s)
22	6.20 (s)	6.28 (br s)	6.06 (br s)	6.06 (br s)	6.18 (br s)
23	7.49 (br s)	7.52 (br s)	7.33 (br s)	7.33 (br s)	7.37 (br s)
28	2.42 (s, 3H)	2.44 (s, 3H)	1.78 (s, 3H)	1.58 (s, 3H)	1.60 (s, 3H)
29				1.52 (s, 3H)	1.45 (s, 3H)
30	1.91 (s, 3H)	1.67 (s, 3H)	1.36 (s, 3H)	1.39 (s, 3H)	1.34 (s, 3H)
2'	2.07 (m, 2H)	2.00 (m, 2H)	2.24 (q, 7.5, 2H)	2.28 (m, 2H)	
3'	0.86 (t, 7.5, 3H)	0.85 (t, 7.5, 3H)	1.04 (t, 7.6, 3H)	1.08 (t, 7.6, 3H)	
OMe-1			3.27 (s, 3H)		
OAc-11		2.18 (s, 3H)			
OMe-29	3.82 (s, 3H)	3.86 (s, 3H)	3.73 (s, 3H)		

[a] Measured in C_5D_5N at 400 MHz.
[b] Measured in C_5D_5N at 600 MHz.
[c] Measured in $CDCl_3$ at 600 MHz.

compound **6** was the 1α-hydroxy-1,2-dihydro derivative of **5**. Thus, compound **6** was therefore established as 1α-hydroxy-1,2-dihydrodeacetylhirtin.

Compound **7**, which was in the form of colourless crystals, had the molecular formula $C_{32}H_{38}O_{12}$ as determined by the HR-ESI(+) MS ion peak at *m*/*z* 637.2259 $[M+Na]^+$ (calcd 637.2255). An analysis of its NMR data (Tables 2 and 3) indicated that compound **7** possessed the same structure as **6**. The primary difference was the presence of one additional acetyl (δ_H 2.18; δ_C 21.6, 170.3) group. The acetyl group is located at C-11 according to the ^1H–^{13}C HMBC correlation between H-11 (δ_H 5.87) and OAc-11 (δ_C 170.3) (see Supporting information). Thus, **7** was identified as 1α-hydroxy-1,2-dihydrohirtin.

Compound **8** was isolated in the form of colourless crystals. Its molecular formula of $C_{31}H_{38}O_{11}$ was determined by the HR-ESI(+) MS ion peak at *m*/*z* 587.2491 $[M+Na]^+$ (calcd 587.2487). A comparison of the NMR data (Tables 2 and 3) for **8** with those of **6** indicated that they were structural analogues. Their major differences were the resonances of an extra methoxy (δ_H 3.27; δ_C 56.5) group in **8** that replaced the 1-hydroxy in **6**. These structural differences were also confirmed by the ^1H-^{13}C HMBC correlation observed between H-1 at δ_H 3.68 (d, *J* = 5.7) and OMe-1, and the ^1H–^1H ROESY correlation between protons H-19 and H-1 showed that the methoxy group was α-oriented (see Supporting information). The structure of **8** was thereby established and named 1α-methoxy-1,2-dihydrodeacetylhirtin.

Compound **9** displayed an ion peak at *m*/*z* 533.2157 $[M+Na]^+$ in the HR-ESI(+)MS spectrum, which was consistent with a molecular formula of $C_{29}H_{34}O_8$ (calcd 533.2146). An analysis of the NMR data (Tables 2 and 3) indicated that **9** was also a cedrelone limonoid. Compared with 11β-hydroxycedrelone, compound **9** incorporated an additional 12-propanoyl side-chain at δ_H 1.08 (t, *J* = 7.6), 2.28 (m) and δ_C 9.2, 28.0, 174.0 instead of the 12-methylene unit (Luo et al., 2000), which was confirmed by the ^1H-^{13}C HMBC correlation between H-12 (δ_H 5.1) and C-1′ (δ_C 174.0) and the ^1H–^1H COSY correlation between H_3-3′ at δ_H 1.08 (t, *J* = 7.6) and H-2′ at δ_H 2.28 (m). The relative configuration of H-12 was assigned to a β-configuration according to its NOE interaction with H-17β at δ_H 2.93 (dd, *J* = 11.0, 6.6) (see Supporting information). Therefore, the structure of **9** was identified and named 11β-hydroxy-12α-propanoyloxycedrelone.

The molecular formula ($C_{26}H_{32}O_7$) of compound **10** was established via the HR-ESI(−)MS ion peak at *m*/*z* 455.2078 (calcd 455.2075), representing 16 mass units more than that of 11β-hydroxydihydrocedrelone (Luo et al., 2000). The NMR data (Tables 2 and 3) were identical to those of 11β-hydroxydihydrocedrelone, with the marked difference being the presence of an additional hydroxyl group. The downfield shift in signal for the oxymethine (C-1, from δ_C 35.8 to 70.9) suggested that this hydroxyl group was located at C-1, which was further confirmed by the ^1H–^{13}C HMBC correlations H-1/C-3, H-9/C-1, and H-19/C-1. The ^1H–^1H ROESY correlation between the proton signals of H-19 and H-1 indicated that the hydroxyl group was α-oriented (see Supporting information). Thus, **10** was determined as 1α,11β-dihydroxy-1,2-dihydrocedrelone.

Two known limonoids called deacetylhirtin (**11**) and hirtin (**12**) were also identified on the basis of a spectroscopic experiment and

Table 4
Cytotoxic activity (IC_{50} μM) of isolated limonoids against cancer cell lines.

Compound	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	>40	>40	>40	>40	>40
2	>40	>40	>40	>40	>40
3	>40	>40	>40	>40	>40
4	>40	>40	>40	>40	>40
5	4.9	3.1	2.9	9.8	9.0
6	3.1	1.0	1.1	1.0	1.6
7	>40	18.0	18.6	39.6	33.3
8	5.3	3.7	5.2	10.2	15.9
9	14.8	5.3	6.4	15.4	15.7
10	>40	20.6	18.5	>40	>40
11	0.4	0.1	0.1	0.5	0.3
12	0.1	0.1	0.1	0.3	0.1
Cisplatin	1.1	5.7	8.5	13.5	17.3

a comparison of their spectroscopic data with those in the literature (Simmonds et al., 2001).

All isolated limonoids were evaluated for their cytotoxic activity against human myeloid leukaemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW480) cell lines by MTS assay. Compounds **11** and **12** showed significant cytotoxicity with IC_{50} values ranging from 0.1 to 0.5 μM, compounds **5**, **6**, **8**, and **9** exhibited potent cytotoxic activity with IC_{50} values ranging from 1.0 to 15.9 μM, and compounds **7** and **10** displayed selective cytotoxicity with IC_{50} values ranging from 18.5 to 39.6 μM, whereas other limonoids were inactive (Table 4) and comparable to the cisplatin positive control (IC_{50}: 1.1–17.3 μM).

3. Conclusions

The phytochemical investigation of the *T. americana* extract yielded 10 new limonoids (**1**–**10**); there were also two previously known limonoids (**11**–**12**). All the isolated limonoids were biologically evaluated for their cytotoxic activity against five human tumour cell lines. Compound **11**, a major limonoid in this species, exhibited significant cytotoxicity with IC_{50} values ranging from 0.1 to 0.5 μM. This study is the first report on the chemical constituents of *T. americana* and the enriched diversity of limonoids in the genus *Trichilia*.

4. Experimental section

4.1. General

Melting points were obtained on an X-4 digital micromelting point apparatus and are uncorrected. Optical rotations were obtained with a JASCO P-1020 polarimeter. UV spectra were measured with a Shimadzu UV-2401A instrument. IR spectra (KBr) were determined on a Bruker Tensor-27 infrared spectrometer. The NMR spectra were recorded on Bruker AM-400, and Bruker Avance III 600 spectrometers with TMS as an internal standard. HR-ESIMS were recorded on a Q-TOF Micro LC–MS-MS mass spectrometer. Semipreparative HPLC was performed on a Waters 600 pump system with a 2996 photodiode array detector by using a YMC-Pack ODS-A column (300 × 10 mm, S-5 μm). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd), C_{18} reversed-phase silica gel (150–200 mesh, Merck), Sephadex LH-20 gel (40–70 μm, Amersham Pharmacia Biotech Ltd), and MCI gel (CHP20/P120, 75–150 μm, Mitsubishi Chemical Industries Ltd) were used for column chromatography (CC). Pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Co. Ltd) were used for analytical TLC.

4.2. Plant material

Leaves of *T. americana* were collected from XTBG, Mengla Country, Yunnan Province in the People's Republic of China in March 2013, and they were identified by one of the authors (C.-F. Xiao). A voucher specimen (HITBC_028844) is deposited in the herbarium at XTBG.

4.3. Extraction and isolation

Air-dried leaves (5.0 kg) were extracted with EtOH/H_2O (30 L, 95:5, v/v) three times (for seven days each time) at room temperature. Removal of the solvent from the combined extracts in vacuo afforded a crude residue (250 g). The latter was suspended in H_2O (1 L) and partitioned with EtOAc. The EtOAc-soluble fraction (90 g) was subjected to silica gel CC and eluted with a gradient of petroleum ether/acetone (50:1 to 0:1) to produce five fractions (1–5). Fraction 2 (10.8 g) was applied to an MCI gel column with MeOH/H_2O (1:1 to 4:1) to yield **10** (8 mg), **11** (180 mg), and **12** (20 mg). Fraction 3 (15 g) was separated over a MCI gel column with MeOH/H_2O (2:3 to 7:3) to produce four further fractions (3A–3D), and fraction 3B was subjected to Sephadex LH-20 gel CC with MeOH/H_2O (2:3 to 3:2) to obtain three major subfractions, namely 3B1, 3B2, and 3B3. Subfraction 3B2 was further purified by semi-preparative HPLC with MeOH/H_2O (1:1 to 4:1) to yield **7** (2 mg), **8** (3 mg), and **9** (3 mg). Fraction 3C was separated using silica gel CC with petroleum ether/acetone (5:1 to 1:2) to yield **5** (10 mg) and **6** (20 mg). Fraction 4 (8 g) was fractionated on a reversed-phase C_{18} silica gel column with MeOH/H_2O (3:7 to 3:2) to give three further fractions (4A–4C). Fraction 4B was then purified by semi-preparative HPLC with MeOH/H_2O (3:7 to 1:1) to yield **1** (10 mg), **2** (3 mg), and **3** (4 mg); and a similar procedure was employed to fraction 4C to yield **4** (5 mg).

4.3.1. Americanolide A (**1**)

Colourless crystals; mp 191–193 °C; $[\alpha]_D^{23.6}$ + 50.2 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.01), 276 (3.90), and 343 (3.09) nm; IR (KBr) ν_{max} 3433, 3113, 3105, 2922, 2852, 1755, 1735, 1680, 1635, 1462, 1373, 1264, 1205, 1088, and 1039 cm^{-1}; positive HRESIMS *m*/*z* 641.2200 $[M+Na]^+$ (calcd for $C_{31}H_{38}O_{13}Na$, 641.2205); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.2. Americanolide B (**2**)

Colourless crystals; mp 198–200 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.38), 276 (4.13), and 342 (3.42) nm; IR (KBr) ν_{max} 3441, 2925, 1731, 1632, 1384, 1205, 1087, and 1036 cm^{-1}; positive HRESIMS *m*/*z* 655.2365 $[M+Na]^+$ (calcd for $C_{32}H_{40}O_{13}Na$, 655.2361); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.3. Americanolide C (**3**)

Colourless crystals; mp 114–116 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.58), 275 (4.21), and 344 (3.60) nm; IR (KBr) ν_{max} 3441, 2926, 1731, 1632, 1384, 1204, 1085, and 1038 cm^{-1}; positive HRESIMS *m*/*z* 623.2103 $[M+Na]^+$ (calcd for $C_{31}H_{36}O_{12}Na$, 623.2099); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.4. Americanolide D (**4**)

Colourless crystals; mp 178–180 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.96), 275 (3.83), and 344 (3.05) nm; IR (KBr) ν_{max} 3440, 2921, 2852, 1734, 1644, 1463, 1384, 1266, 1207, 1090, and 1040 cm^{-1}; positive HRESIMS *m*/*z* 627.2058 $[M+Na]^+$ (calcd for $C_{30}H_{36}O_{13}Na$, 627.2048); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.5. 1,2-Dihydrodeacetylhirtin (**5**)

Colourless crystals; mp 115–117 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.84), 275 (3.80), and 344 (3.15) nm; IR (KBr) ν_{max} 3431, 2987, 2949, 1740, 1716, 1681, 1637, 1504, 1462, 1379, 1354, 1271, 1247, 1206, 1128, 1089, and 1039 cm^{-1}; positive HRESIMS *m*/*z* 557.2400 $[M+H]^+$ (calcd for $C_{30}H_{37}O_{10}$, 557.2381); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.6. 1α-Hydroxy-1,2-dihydrodeacetylhirtin (**6**)

Colourless crystals; mp 142–144 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.83), 263 (3.75), 276 (3.78), and 345 (3.20) nm; IR (KBr) ν_{max} 3439, 2987, 2950, 1736, 1681, 1639, 1504, 1461, 1378, 1352, 1257, 1206, 1132, 1086, and 1039 cm^{-1}; positive HRESIMS *m*/*z* 573.2342 $[M+H]^+$ (calcd for $C_{30}H_{37}O_{11}$, 573.2330); for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

*4.3.7. 1α-Hydroxy-1,2-dihydrohirtin (**7**)*

Colourless crystals; mp 204–206 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.50), 275 (3.10), and 344 (2.61) nm; IR (KBr) ν_{max} 3433, 2928, 1742, 1632, 1384, 1238, and 1038 cm^{-1}; positive HRESIMS *m/z* 637.2259 $[M+Na]^+$ (calcd for $C_{32}H_{38}O_{12}Na$, 637.2255); for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

*4.3.8. 1α-Methoxy-1,2-dihydrodeacetylhirtin (**8**)*

Colourless crystals; mp 144–146 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.82), 276 (3.79), and 344 (3.22) nm; IR (KBr) ν_{max} 3432, 2987, 2950, 1738, 1715, 1681, 1640, 1504, 1461, 1353, 1253, 1213, 1133, 1085, 1041, and 1023 cm^{-1}; positive HRESIMS *m/z* 587.2491 $[M+H]^+$ (calcd for $C_{31}H_{39}O_{11}$, 587.2487); for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

*4.3.9. 11β-Hydroxy-12α-propanoyloxycedrelone (**9**)*

Colourless crystals; mp 134–136 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.19), and 279 (3.97) nm; IR (KBr) ν_{max} 3438, 2925, 1680, 1631, 1384, 1189, and 1037 cm^{-1}; positive HRESIMS *m/z* 533.2157 $[M+Na]^+$ (calcd for $C_{29}H_{34}O_8Na$, 533.2146); for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

*4.3.10. 1α,11β-Dihydroxy-1,2-dihydrocedrelone (**10**)*

Colourless crystals; mp 126–128 °C; $[\alpha]_D^{27}$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.76), 279 (3.84), and 349 (3.04) nm; IR (KBr) ν_{max} 3420, 2924, 1677, 1626, 1383, 1352, 1254, 1123, and 1035 cm^{-1}; negative HRESIMS *m/z* 455.2078 $[M-H]^-$ (calcd for $C_{26}H_{31}O_7$, 455.2075); for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

4.4. X-ray diffraction analysis

Crystal data of **1**, Orthorhombic, $C_{31}H_{38}O_{13}\cdot H_2O$, space group *P*212121 with *a* = 13.5659(9) Å, *b* = 15.0473(9) Å, *c* = 29.761(2) Å, and *V* = 6075.0(7) $Å^3$; *Z* = 8; D_c = 1.392 Mg m^{-3}; and *F* (000) = 2704. Crystal size: 0.52 × 0.43 × 0.07 mm^3. Independent reflections 10270 [*R*(int) = 0.1672)]. The final indices were R_1 = 0.1107 and wR_2 = 0.2891 [$I > 2\sigma(I)$]. The final indices were R_1 = 0.1707 and wR_2 = 0.3623 (all data). The goodness of fit on F^2 was 1.123. The X-ray crystallographic data were obtained on a Bruker APEX DUO CCD diffractometer equipped with graphite monochromated Cu-Kα radiation (λ = 1.54178 Å) at 100 (2) K. The structure was solved by direct method with SHELXS-97 (Sheldrick, 2008) and refined with full-matrix least-squares calculations on F^2 by using SHELXS-97 (Sheldrick, 2008). All non-hydrogen atoms were refined anisotropically. The hydrogen atom position were geometrically idealized and allowed to ride on their parent atoms. The crystallographic data for structure **1** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-1044160. Those data can be obtained free of charge from the CCDC, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; and e-mail: deposit@ccdc.cam.ac.uk.

4.5. Cytotoxic assay

The cytotoxic activities against human myeloid leukaemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW480) cell lines were tested by MTS assay as previously reported (Ji et al., 2014a).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2015.08.014.

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