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Structural elucidation of limonoids and steroids from *Trichilia connaroides*

Xiao-Ning Wang, Cheng-Qi Fan, Sheng Yin, Li-She Gan, 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

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Abstract

Six limonoids, trijugins D–H (**1–5**) and methyl 8α-hydroxy-8,30-dihydroangolensate (**6**), two degraded limonoids, trichiconnarins A and B (**7–8**), and a pregnane steroid, 3β,4α-dihydroxypregnan-21-one (**9**), along with the known trijugin C (**10**) and 3β,4α-dihydroxypregnan-16-one (**11**) were isolated from twigs and leaves of *Trichilia connaroides*. Their structures were established on the basis of extensive spectroscopic analysis.

Keywords: *Trichilia connaroides*; Meliaceae; Limonoids; Trijugins; Trichiconnarins; Pregnane

1. Introduction

The structurally diversified limonoids with significant biological activities from plants of the Meliaceae family have been attracting considerable interest (Taylor, 1984; Champagne et al., 1992; Mulholland et al., 2000; Roy and Saraf, 2006). *Trichilia connaroides* (Wight et Arn.) Bentv. (previous name: *Heynea trijuga* Roxb.) (Meliaceae) is a tall tree widely distributed in the South and East of Asia, such as India, Indonesia, and South China (Chen et al., 1997). Previously, three triterpenoids, four limonoids, and a steroid have been characterized from the leaves, flowers, roots, or pericarps of this plant (Purushothaman et al., 1983, 1987; Venkatanarasimhan and Kundu, 1990; Inada et al., 1994; Zhang et al., 2003). In the present research, six new limonoids, trijugins D–H (**1–5**) and methyl 8α-hydroxy-8,30-dihydroangolensate (**6**), two new degraded products of limonoids, trichiconnarins A and B (**7–8**), and a new pregnane steroid, 3β,4α-dihydroxypregnan-21-one (**9**), along with the known trijugin C (**10**) and 3β,4α-dihydroxypregnan-16-one (**11**) were isolated from the twigs and leaves of *T. connaroides*. Herein we reported the isolation and structure elucidation of the new compounds.

2. Results and discussion

Trijugin D (**1**) was obtained as a white amorphous powder. The HREIMS displayed a molecular ion peak at *m/z* 544.1946, consistent with a molecular formula of $C_{28}H_{32}O_{11}$ (calcd 544.1945). The strong IR absorption bands at 1799, 1763, 1747, 1724, and 1703 cm^{-1} indicated the presence of several carbonyl functionalities. The ^{1}H and ^{13}C NMR spectroscopic data in combination with the HMBC experiment indicated that **1** was very similar to trijugin C (**10**) (Zhang et al., 2003), except for the presence of an additional acetyl group. The acetoxyl group was then attached to C-8 based on the observations that the exchangeable sharp singlet for OH-8 (δ_H 5.87) in **10** disappeared, and that the C-8 resonance was deshielded in **1** (δ_C 89.6) as compared with that in **10** (δ_C 87.6 in $CDCl_3$ and 88.0 in pyridine-d_5) (Zhang et al., 2003). The similar NOESY correlation patterns as well as the similar ^{1}H

* Corresponding author. Tel./fax: +86 21 50806718.
E-mail address: jmyue@mail.shcnc.ac.cn (J.-M. Yue).

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and ^{13}C NMR spectroscopic data to those of trijugin C (**10**) suggested that **1** shared the same relative stereochemistry with **10**. Therefore, trijugin D (**1**) was determined to be 8-*O*-acetyltrijugin C.

Trijugin E (**2**) was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{28}H_{32}O_{12}$ by the HREIMS ($[M]^+$ at *m/z* 560.1889, calcd 560.1894) and NMR data. The IR, ^1H and ^{13}C NMR spectroscopic data (Table 1) of **2** were very similar to those of **1**, and the only difference was the presence of an additional hydroxyl at C-2 in **2** as judged from the absence of the H-2 resonance and the appearance of an exchangeable sharp singlet at δ_H 5.83 (OH-2) in the ^1H NMR spectrum. The above conclusion was verified by the HMBC correlations of OH-2/C-1 (δ_C 82.3), C-2, and C-8; H-1/C-2; and H-11β/C-2. The correlations of OH-2 with H-1 and H-11β in the NOESY spectrum indicated that OH-2 was β-oriented. The stereochemistry at other chiral centers was determined to be the same as that of **1** by the NOESY experiment. Trijugin E (**2**) was thus established to be 2β-hydroxytrijugin D.

Trijugin F (**3**) was also obtained as a white amorphous powder. The molecular formula was determined to be $C_{26}H_{30}O_{10}$ by the HREIMS ($[M]^+$ at *m/z* 502.1835, calcd 502.1839) and NMR data. The ^1H and ^{13}C NMR spectroscopic data of **3** (Table 1) were very similar to those of **10**, indicating that they were structurally related analogs. In comparison, the proton resonance of H-17 (δ_H 5.37, *d*, $J = 3.5$ Hz) and the carbon resonance of C-17 (δ_C 69.7) in **3** were obviously up-field shifted as referred to those in **10** (δ_H 5.98, *s*; δ_C 81.1 for CH-17), and an exchangeable doublet assigned as OH-17 was observed at δ_H 6.87 (*d*, $J = 3.5$ Hz). In addition, the resonances for C-8 (δ_C 102.0), C-14 (δ_C 100.2), C-15 (δ_C 41.0), and C-16 (δ_C 175.6) in **3** were all considerably deshielded as compared to those in **10** (δ_C 88.0, 93.4, 37.7, and 168.9, respectively). The above evidence suggested that the six-membered ring-D δ-lactone of **10** was hydrolyzed, and a new five-membered γ-lactone ring was formed between C-16 and C-8. The NOESY correlations of OH-17/H-17, H-21, and H-22 confirmed that the hydroxyl was present at C-17. The NOESY correlations (Fig. 1) of H-15b/H-5 and H-15a/

Table 1
^1H and ^{13}C NMR spectroscopic data for compounds **1–3** and **10**[a]

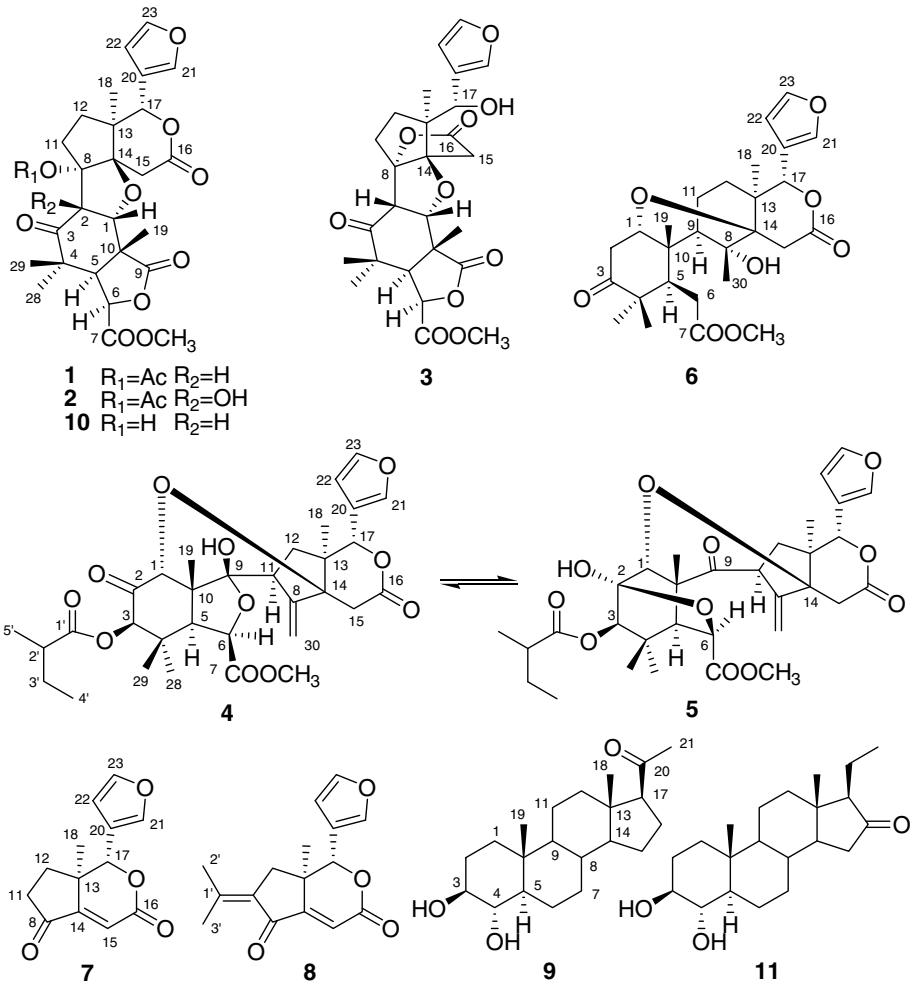
Position	**1**[b]		**2**[b]		**3**[c]		**10**[c]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.42 (*d*, 4.2)	80.0	4.18 (*s*)	82.3	5.14 (*d*, 7.2)	84.9	4.72 (*d*, 4.7)	80.2
2	3.60 (*d*, 4.2)	51.6		78.8	3.90 (*d*, 7.2)	62.0	3.56 (*d*, 4.7)	55.8
3		208.0		210.0		209.4		215.3
4		47.7		47.6		47.4		47.9
5	3.39 (*d*, 8.7)	45.2	3.32 (*d*, 8.7)	45.5	3.88 (*d*, 8.5)	46.7	3.84 (*d*, 8.7)	46.0
6	5.05 (*d*, 8.7)	74.4	5.04 (*d*, 8.7)	74.3	5.47 (*d*, 8.5)	75.2	5.46 (*d*, 8.7)	74.8
7		170.0		169.9		171.3		170.9
8		89.6		95.2		102.0		88.0
9		175.4		175.4		176.8		176.5
10		43.0		43.2		44.9		44.0
11α	2.42 (*m*, 2H)	41.7	2.07 (*m*)	34.4	2.10 (*m*)	38.3	2.24 (*m*)	43.4
11β			3.27 (*m*)		2.45 (*m*)		2.47 (*m*)	
12α	1.58 (*m*)	33.4	1.66 (*m*)	33.2	1.30 (*m*)	36.6	1.44 (*m*)	33.4
12β	1.87 (*m*)		1.94 (*m*)		1.99 (*m*)		1.83 (*m*)	
13		47.9		48.7		51.9		48.3
14		92.9		91.5		100.2		93.4
15α	2.96 (*d*, 18.0)	36.8	2.97 (*d*, 18.0)	37.0	a 3.48 (*d*, 19.6)	41.0	3.34 (*d*, 18.1)	37.7
15β	2.25 (*d*, 18.0)		2.24 (*d*, 18.0)		b 3.25 (*d*, 19.6)		2.70 (*d*, 18.1)	
16		168.3		167.9		175.6		168.9
17	5.69 (*s*)	80.7	5.71 (*s*)	79.8	5.37 (*d*, 3.5)	69.7	5.98 (*s*)	81.1
18	0.95 (*s*, 3H)	15.8	0.97 (*s*, 3H)	15.9	1.13 (*s*, 3H)	18.8	1.13 (*s*, 3H)	15.9
19	1.58 (*s*, 3H)	18.7	1.70 (*s*, 3H)	16.4	1.57 (*s*, 3H)	19.4	1.47 (*s*, 3H)	18.8
20		121.3		121.2		128.4		122.7
21	7.43 (*s*)	139.9	7.44 (*s*)	140.0	7.70 (*br s*)	135.9	7.64 (*br s*)	140.5
22	6.28 (*s*)	108.4	6.29 (*s*)	108.4	6.60 (*s*)	110.9	6.40 (*d*, 1.0)	109.4
23	7.41 (*s*)	143.3	7.42 (*s*)	143.4	7.60 (*s*)	140.8	7.60 (*t*, 1.6)	143.8
28	1.40 (*s*, 3H)	26.2	1.44 (*s*, 3H)	25.8	1.54 (*s*, 3H)	27.5	1.71 (*s*, 3H)	25.7
29	1.07 (*s*, 3H)	21.9	1.26 (*s*, 3H)	23.0	1.22 (*s*, 3H)	22.5	1.17 (*s*, 3H)	21.5
OMe	3.84 (*s*, 3H)	52.8	3.86 (*s*, 3H)	52.7	3.68 (*s*, 3H)	52.8	3.74 (*s*, 3H)	52.7
8-OAc		170.4		173.6				
	2.19 (*s*, 3H)	21.6	2.21 (*s*, 3H)	21.4				
OH			5.83 (*s*)		6.87 (*d*, 3.5)			

[a] Recorded at 400 and 100 MHz for ^1H and ^{13}C NMR, respectively. δ in ppm and *J* in Hz are in the parentheses.
[b] In $CDCl_3$.
[c] In pyridine-d_5.

H_3-18 indicated that the hemisphere (C-15, C-16 and O-8) of the five-membered γ-lactone was in the α-face of **3**, and the stereochemistry at C-8 and C-14 was the same as that in **10**. The steric bulk considerably fixed the free rotation along the C-13 and C-17 bond, and allowed the observation of the mutual NOESY correlations of H-12β/H-17, H-1, H-2; H-17/H-1; and H-2/H-11β, indicating that they were all β-oriented. Compound **3** was likely derived from **10** via an intramolecular ester-interchange reaction, in which, the stereochemistry of C-17 was retained. This was supported by the fact that a number of ring-D opened limonoids with a retained stereochemistry of C-17 were reported (Saad et al., 2003; Hay et al., 2007; Chen et al., 2007). The structure of trijugin F (**3**) was fully elucidated to be a trijugin C derivative with a new γ-lactone ring.

of the two inseparable compounds were thus elucidated as a mixture. The HREIMS displayed a molecular ion peak at *m/z* 600.2574 that allowed establishment of a common molecular formula $C_{32}H_{40}O_{11}$ for both **4** and **5** (calcd 600.2571). The IR spectrum of the mixture showed absorption bands at 3446 and 1736 cm^{-1} for hydroxyls and carbonyls, respectively. The ^1H and ^{13}C NMR spectroscopic data (Table 2) of **4** and **5** showed high similarity, which indicated the presence of four tertiary methyl, a β-furyl, a carbomethoxyl, an exocyclic double bond, a 2-methylbutanoate moiety, and a ring-D δ-lactone in each compound. The above features suggested that **4** and **5** were two derivatives of trijugin A (Purushothaman et al., 1987).

The HMBC and HSQC spectra (Fig. 2) indicated that the gross structure of **4** differed from trijugin A mainly in



Trijugins G (**4**) and H (**5**) were obtained as a white amorphous solid, which is a mixture of two isomers in equilibrium with a relative ratio of about 1:1 as measured by ^1H NMR. The two compounds were separable by HPLC, but soon after separation, each compound isomerized readily into a mixture of the two hemiketals featuring either the 6,9-oxo (**4**) or 2,6-oxo bridges (**5**). The structures

rings-A and C (Purushothaman et al., 1987). The 12-OAc group in trijugin A was absent, but a 2-methylbutanoate moiety was observed at C-3 (δ_C 81.8) on the basis of HMBC correlations from H-1, H_3-28 (δ_H 1.49, *s*), and H_3-29 (δ_H 1.07, *s*) to C-3, and correlation from H-3 (δ_H 5.69, *s*) to C-1′ (δ_C 175.0). A ketonic carbonyl was assigned to C-2 (δ_C 200.4) as judged from HMBC correlations from

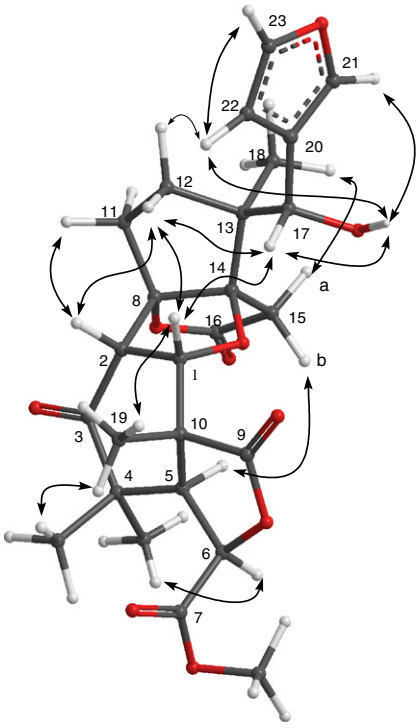


Fig. 1. Key NOESY correlations (H ↔ H) of **3**.

H-1 and H-3 to C-2. The ROESY correlations (Fig. 3) of OH-9 (δ_H 7.80, *s*) with H-11 and H_3-19 suggested that **4** shared the same configuration with that of trijugin A at C-9 (Purushothaman et al., 1987). The correlations of H-3 with H-5, H-17, and H_3-28 in the ROESY spectrum indicated that H-3 was α-oriented. The stereochemistry at other chiral centers in **4** was determined to be the same as that in trijugin A by analysis of the ROESY spectrum.

The HMBC spectrum indicated that trijugin H (**5**) differed from trijugin G (**4**) only in the nature of the hemiketal ring. In the case of **5**, the HMBC correlations (Fig. 2) from H-6 (δ_H 4.97, s) to C-2 (δ_C 97.3) indicated the presence of a 2,6-oxo bridge to form a hemiketal at C-2. The correlations from H-1 (δ_H 4.18, *s*) and H-3 (δ_H 5.37, *s*) to C-2 also confirmed that the hemiketal was present at C-2. The ketone group was placed at C-9 (δ_C 210.9) as judged by the HMBC correlations from H-11 (δ_H 3.79, *m*), H-12β (δ_H 2.49, *m*), and H_3-19 (δ_H 1.47, *s*) to C-9. The ROESY correlations (Fig. 3) between H-6 and H_3-19 suggested that C-6 was in the β-face of ring-A, which in turn indicated that OH-2 was α-oriented. The stereochemistry at other carbons of **5** was also furnished by the ROESY spectrum (Fig. 3).

Methyl 8α-hydroxy-8,30-dihydroangolensate (**6**) was obtained as a white amorphous powder. The HREIMS exhibited a molecular ion peak at *m/z* 488.2403 (calcd 488.2410) corresponding to the molecular formula $C_{27}H_{36}O_8$. The IR spectrum exhibited absorption bands for hydroxyls (3396 cm^{-1}) and carbonyls (1736 and 1716 cm^{-1}). The ^1H and ^{13}C NMR spectroscopic data together with HMBC and HMQC spectra furnished the gross structure of **6**, which is very similar to methyl angolensate (Abdelgaleil et al., 2001). The only difference was that the $\Delta^{8(30)}$ double bond in methyl angolensate was replaced by a hydroxylated quaternary carbon (δ_C 73.4, C-8) and a methyl (δ_C 29.7, C-30), which was verified by the HMBC correlations from H_3-30 to C-8, C-9 (δ_C 48.3), and C-14 (δ_C 77.6). The NOESY correlations of H_3-30/H-15β and H_3-30/H-1 indicated that 30-Me was β-oriented and accordingly, OH-8 was α-oriented. The stereochemistry of the whole molecule was further confirmed by NOESY spectrum.

Trichiconnarin A (**7**) was obtained as a yellowish gum. The molecular formula was determined to be $C_{13}H_{12}O_4$ from the molecular ion peak at *m/z* 232.0732 in the HREIMS (calcd 232.0736). The IR absorption bands at 1728 and 1651 cm^{-1} were ascribable to carbonyls and double bonds. The ^1H and ^{13}C NMR spectra (Table 3) indicated the presence of a tertiary methyl [δ_H 1.14 (*s*, H_3-18); δ_C 17.6], a β-substituted furan ring, and a trisubstituted double bond [δ_H 6.43 (*s*, H-15); δ_C 158.8 (C-14) and 115.8 (C-15)]. The ^{13}C NMR spectrum also displayed two methylenes at δ_C 30.5 (C-12) and 36.4 (C-11), an oxymethine at δ_C 83.0 (C-17), one sp^3 quaternary carbon at δ_C 42.9 (C-13), a conjugated ester carbonyl at δ_C 163.9, and a conjugated ketone carbonyl at δ_C 203.6. Compound **7** was established to be a degraded product of limonoid with a contracted five-membered ring-C, which was demonstrated by the HMBC correlations of H_3-18/C-12, C-13, C-14, and C-17; H-17/C-20, C-21, and C-22; H_2-11/C-8, C-12, C-13, and C-14; and H-15/C-8, C-14, and C-16 (Fig. 4). The unique structural features of **7** suggested that it was likely a degradation product of trijugin C (**10**). Therefore, the stereochemistry of **7** at C-13 and C-17 was assumed to be the same as that of **10**. The correlations of H_3-18/H-12 α and H-17/H-12β in the NOESY spectrum also confirmed the above conclusion.

Trichiconnarin B (**8**) was obtained as a yellow gum. The molecular formula was determined to be $C_{16}H_{16}O_4$ by the HREIMS ($[M]^+$ at *m/z* 272.1048, calcd 272.1049). The IR spectrum exhibited absorption bands for carbonyls (1728 cm^{-1}) and double bonds (1620 cm^{-1}). The ^1H and ^{13}C NMR spectra of **8** (Table 3) were similar to those of **7**, suggesting that they are structurally related analogs. In addition, two geminal tertiary methyls resonating at δ_H 1.94 (*s*, H_3-3′) and 2.38 (*s*, H_3-2′) were correlated to both carbons of a tetrasubstituted double bond at δ_C 130.2 (C-11) and 156.1 (C-1′), which suggested that the two methyls were connected to C-11 through C-1′. The gross structure of **8** was fully determined by the HMBC spectrum (Fig. 4). The stereochemistry of **8** was determined to be the same as that of **7** on the basis of analysis of the NOESY spectrum.

Highly degraded products of limonoids have been encountered in the Rutaceae family, but have rarely been recorded in the Meliaceae (Nakatani et al., 1998; D'Ambrosio and Guerriero, 2002). Trichiconnarins A and B (**7**–**8**) are the first examples of degraded compounds of limonoids with a contracted five-membered ring-C.

Table 2
^{1}H and ^{13}C NMR spectroscopic data for compounds **4–6**[a]

Position	**4**[b]		**5**[b]		**6**[c]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.41 (*s*)	81.8	4.18 (*s*)	81.1	4.09 (*dd*, 9.1, 7.2)	76.5
2		200.4		97.3	α 2.70 (*dd*, 11.9, 7.2) β 2.90 (*dd*, 11.9, 9.1)	41.9
3	5.69 (*s*)	81.8	5.37 (*s*)	76.4		213.5
4		39.7		37.8		48.0
5	3.90 (*d*, 8.8)	53.7	2.89 (*s*)	46.7	3.08 (*d*, 10.0)	46.0
6	5.21 (*d*, 8.8)	75.8	4.97 (*s*)	73.4	α 2.58 (*dd*, 16.4, 10.1) β 2.37 (*d*, 16.4)	33.3
7		174.1		173.6		173.9
8		146.0		146.9		73.4
9		108.2		210.9	1.57 (*m*)	48.3
10		55.0		56.6		42.6
11	3.68 (*m*)	54.4	3.79 (*m*)	54.9	α 2.25 (*m*) β 1.89 (*m*)	18.2
12α	1.83 (*m*)	36.9	2.01 (*m*)	39.0	1.24 (*m*)	29.0
12β	2.57 (*m*)		2.49 (*m*)		1.69 (*m*)	
13		45.8		45.6		41.4
14		89.4		88.3		77.6
15α	3.08 (*d*, 17.9)	34.7	3.13 (*d*, 17.7)	33.0	2.95 (*d*, 18.2)	35.3
15β	3.15 (*d*, 17.9)		3.42 (*d*, 17.7)		2.65 (*d*, 18.2)	
16		168.1		168.1		170.8
17	6.38 (*s*)	79.4	6.33 (*s*)	78.1	5.81 (*s*)	81.2
18	0.79 (*s*, 3H)	18.0	0.82 (*s*, 3H)	19.3	1.20 (*s*, 3H)	15.2
19	1.41 (*s*, 3H)	17.0	1.47 (*s*, 3H)	27.6	0.95 (*s*, 3H)	24.6
20		123.1		122.7		120.9
21	7.65 (*s*)	140.5	7.65 (*s*)	140.4	7.44 (*s*)	140.8
22	6.41 (*s*)	109.2	6.41 (*s*)	109.2	6.39 (*s*)	110.2
23	7.60 (*s*)	144.1	7.58 (s)	143.8	7.41 (*s*)	142.7
28	1.49 (*s*, 3H)	29.4	1.59 (*s*, 3H)	32.0	1.16 (*s*, 3H)	30.2
29	1.07 (*s*, 3H)	17.7	1.10 (*s*, 3H)	24.3	1.20 (*s*, 3H)	20.8
30a	5.68 (*s*)	115.0	5.46 (*s*)	116.1	1.55 (*s*, 3H)	29.7
30b	5.20 (*s*)		5.34 (*s*)			
OMe	3.67 (*s*, 3H)	51.9	3.72 (*s*, 3H)	51.4	3.74 (*s*, 3H)	52.1
1′		175.0		177.0		
2′	2.52 (*m*)	41.2	2.09 (*m*)	40.8		
3′a	1.45 (*m*)	26.9	1.18 (*m*)	25.9		
3′b	1.76 (*m*)		1.47 (*m*)			
4′	0.92 (*t*, 7.5, 3H)	11.7	0.57 (*t*, 7.5, 3H)	11.4		
5′	1.17 (*d*, 7.0, 3H)	16.8	1.12 (*d*, 7.1, 3H)	16.6		
OH	7.80 (*s*)					

[a] Recorded at 400 or 100 MHz for ^{1}H and ^{13}C NMR, respectively. δ in ppm and J in Hz are in the parentheses.
[b] In pyridine-d_5.
[c] In $CDCl_3$.

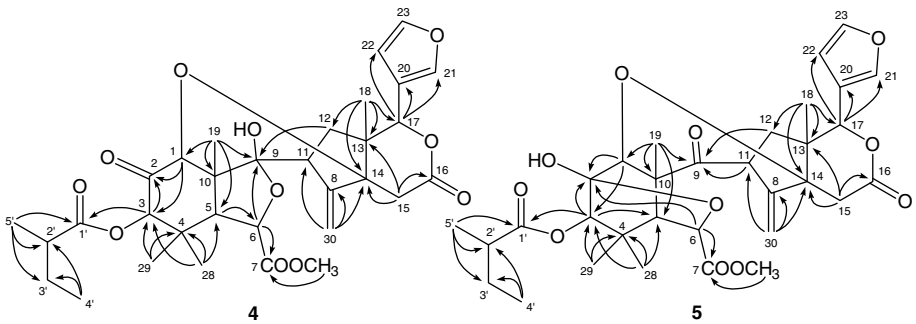


Fig. 2. Key HMBC correlations (H → C) of **4** and **5**.

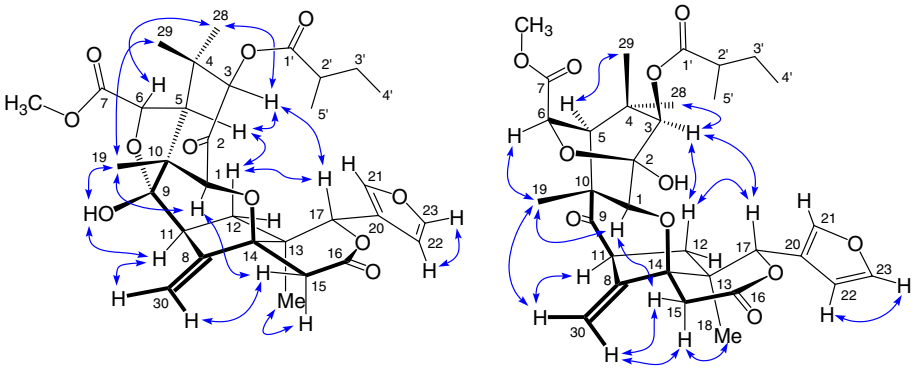


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Fig. 3. Key ROESY correlations (H ↔ H) of **4** and **5**.

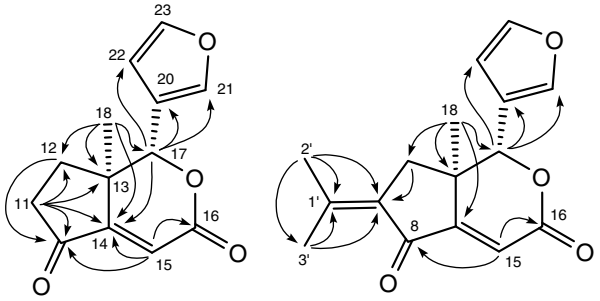
Table 3
^{1}H and ^{13}C NMR spectroscopic data for compounds **7** and **8**[a]

Position	**7**		**8**	
	δ_H	δ_C	δ_H	δ_C
8		203.6		192.0
11	2.62 (*m*, 2H)	36.4		130.2
12α	2.00 (*m*)	30.5	2.52 (*s*, 2H)	37.8
12β	1.92 (*m*)			
13		42.9		39.8
14		158.8		162.9
15	6.43 (*s*)	115.8	6.42 (*s*)	114.9
16		163.9		164.3
17	5.33 (*s*)	83.0	5.33 (*s*)	82.0
18	1.14 (*s*, 3H)	17.6	1.09 (*s*, 3H)	19.5
20		120.0		120.3
21	7.58 (*s*)	140.4	7.59 (*s*)	140.5
22	6.43 (*s*)	108.4	6.47 (*s*)	108.6
23	7.49 (*s*)	143.8	7.49 (*s*)	143.7
1′				156.1
2′			2.38 (*s*, 3H)	21.5
3′			1.94 (*s*, 3H)	24.8

[a] Recorded in $CDCl_3$ at 400 and 100 MHz for ^{1}H and ^{13}C NMR, respectively. δ in ppm and J in Hz are in the parentheses.

Compounds **7** and **8** were likely to be the degradation products of trijugin C by cleavage of the C-2 and C-8 bond (Drews et al., 1985). Compound **8** may be derived from **7** and acetone through an aldol reaction followed by dehydration. As compound **8** was obtained in very limited amount, it is probably an artifact during the extraction and isolation process.

Compound **9** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{21}H_{34}O_3$ by the HREIMS ($[M]^+$ at *m/z* 334.2514, calcd 334.2508). The IR spectrum showed absorption bands at 3386 and 1705 cm^{-1} ascribable to hydroxyl and carbonyl functionalities, respectively. The ^{1}H and ^{13}C NMR spectroscopic data together with the ^{1}H–^{1}H COSY, HMBC, and HMQC spectra indicated that **9** shared the same A, B, and C rings as the known compound 3β,4α-dihydroxypregnan-16-one (**11**) (Zhang et al., 2003). However, instead of the C-16 ketone group in **11**, a ketone group was located at C-20 in **9** by the HMBC correlations from H_3-21 (δ_H 2.10, *s*) to C-17 (δ_C 63.8) and C-20 (δ_C 209.7) and correlations from H-17 (δ_H 2.50, *m*) to C-16 (δ_C 22.8) and C-20. The relative stereochemistry of **9** was determined to be the same as that of **11** by the NOESY spectrum, which was supported by its very similar ^{13}C NMR spectroscopic data to that of **11**. Therefore, compound **9** was elucidated as 3β,4α-dihydroxypregnan-21-one.



7 8

Fig. 4. Key HMBC correlations (H → C) of **7** and **8**.

3. Conclusion

In conclusion, the structures of nine new compounds from *T. connaroides* were identified. Among them, **1–6** are B,D-*seco* limonoids, especially **1–5**, in which ring-C contracted to a five-membered ring. Compounds **7** and **8** are rare degraded limonoids. The discovery of **1–8** is an important addition to the diverse and complex class of limonoids. Plants of the Meliaceae family proved to be a rich source of limonoids. A series of novel limonoids with different carbon skeletons have been isolated recently (Zhang et al., 2007; Yin et al., 2006, 2007; Hay et al., 2007; Wang et al., 2006, 2007; Abdelgaleil et al., 2006; Cui et al., 2005). Previous investigations on other species of the genus *Trichilia* have led to the isolation of some dozens of limonoids belonging to the structural groups of havanensins (A,B,C,D-intact), prieurianins (A,B-*seco*), evodulones (A-*seco*), methyl ivorensates (A,B,D-*seco*),

and obacunols (A,D-*seco*) (Mulholland et al., 2000; Garcez et al., 1997, 2000). However, quite different from those species, the present study and previous reports on *T. connaroides* elaborated none of the above structural types but the andirobin (B,D-*seco*), mexicanolide (B-*seco* and recyclized and D-*seco*), and trijugin (B,D-*seco* and C-contracted) structural classes (Purushothaman et al., 1983, 1987; Venkatanarasimhan and Kundu, 1990; Inada et al., 1994; Zhang et al., 2003), suggesting that the biosynthetic pathway in this species is probably different from that in the other species of this genus. The structural differences can be used as the chemical marks to differentiate *T. connaroides* from the other species of this genus.

4. Experimental

4.1. General

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. UV spectra were obtained on a Shimadzu UV-2550 spectrophotometer. IR spectra were obtained on a Perkin–Elmer 577 spectrometer with 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. int.) 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 CC. Pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China) were used for TLC.

4.2. Plant material

The twigs and leaves of *T. connaroides* were collected in August, 2005 from Xishuangbanna area of Yunnan Province, People's Republic of China, and were authenticated by Prof. Y.-K. Xu, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, People's Republic of China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, SIBS, Chinese Academy of Sciences (accession number: TC-2005-1Y).

4.3. Extraction and isolation

The air-dried powder of the plant (4.4 kg) was percolated with 95% EtOH, and the crude extract (120 g) was subsequently extracted with EtOAc. The EtOAc-soluble fraction (65 g) was separated by MCI-gel CC eluted with a gradient of MeOH–H_2O (0:100 → 100:0) to give six fractions (A–F). Fraction D (10 g) was then separated on a silica-gel column eluted with petroleum ether–Me_2CO (100:0 → 0:100) to give six fractions (D1–D6). Fraction D3 (3.20 g) was applied to a silica-gel column ($CHCl_3$–MeOH, 100:1 → 50:1) to give four parts (D3a–D3d). Fraction D3a (30 mg) was subjected to CC of silica gel (petroleum ether–Me_2CO, 10:1) to give the major part, and then separated by preparative silica gel TLC (petroleum ether–Me_2CO, 10:1) to afford **7** (7.6 mg) and **8** (3.1 mg). Fraction D3b (2.30 g) was separated by silica gel CC (petroleum ether–Me_2CO, 10:1) into five major parts (D3b1–D3b5). Part D3b1 (0.32 g) was successively separated by silica gel (CH_2Cl_2–MeOH, 50:1) and Sephadex LH-20 (EtOH) CC to afford **2** (120 mg). Part D3b2 (1.20 g) was subjected to silica-gel CC (CH_2Cl_2–MeOH, 50:1) to give two parts. The first part (0.60 g) was separated by preparative HPLC (CH_3CN–H_2O, 70:30, 3 ml/min) to yield **10** (60 mg) and **1** (200 mg). The other part (0.50 g) was subjected to silica gel CC (petroleum ether–Me_2CO, 4.5:1) and then preparative HPLC (H_3CN–H_2O, 70:30, 3 ml/min) to afford **3** (310 mg), **4**, and **5**. Both **4** and **5** isomerized very quickly and were later combined together (30 mg) for structure elucidation. Part D3b3 (0.30 g) was subjected to reversed-phase C_{18} silica-gel CC (MeOH–H_2O, 50:50 → 70:30) to collect three major parts, each of which was further purified by Sephadex LH-20 CC (EtOH) to afford **6** (20 mg), **11** (35 mg), and **9** (18 mg).

4.4. Trijugin D (*1*)

White amorphous powder; $[\alpha]_D^{20}$ − 39.0 (*c* 0.110, $CHCl_3$); UV (MeOH), no maxima above 210 nm; IR (KBr) ν_{max} cm^{-1}: 2976, 1799, 1763, 1747, 1724, 1703, 1371, 1244, 1196, 1051, 600; EIMS *m/z* (rel. int.): 544 $[M]^+$ (100), 502 (28), 485 (6), 406 (12), 388 (25), 370 (7), 342 (12), 198 (33), 137 (36), 109 (25), 95 (40); ESIMS (positive mode) *m/z* (rel. int.): 485 $[M+H–CH_3COOH]^+$ (50), 567 $[M+Na]^+$ (18), 1089 $[2M+H]^+$ (52), 1111 $[2M+Na]^+$ (100); ESIMS (negative mode) *m/z* (rel. int.): 483 $[M–H–CH_3COOH]^-$ (89), 543 $[M–H]^-$ (100); HREIMS *m/z* 544.1946 $[M]^+$ (calcd for $C_{28}H_{32}O_{11}$, 544.1945); for ^1H and ^{13}C NMR spectroscopic data, see Table 1.

4.5. Trijugin E (*2*)

White amorphous powder; $[\alpha]_D^{20}$ − 32.0 (*c* 0.115, $CHCl_3$); UV (MeOH), no maxima above 210 nm; IR (KBr) ν_{max} cm^{-1}: 3384, 2979, 1797, 1751, 1722, 1371, 1271, 1228, 1176, 1041, 875, 602; EIMS *m/z* (rel. int.): 560 $[M]^+$ (100), 473 (4), 275 (6), 198 (48), 137 (56), 109 (22), 95 (18); HREIMS *m/z* 560.1889 $[M]^+$ (calcd for $C_{28}H_{32}O_{12}$, 560.1894); for ^1H and ^{13}C NMR spectroscopic data, see Table 1.

4.6. Trijugin F (*3*)

White amorphous powder; $[\alpha]_D^{20}$ − 32.0 (*c* 0.120, $CHCl_3$); UV (MeOH), no maxima above 210 nm; IR (KBr) ν_{max}

cm^{-1}: 3519, 2974, 1790, 1749, 1705, 1469, 1336, 1219, 1118, 1074, 968, 873, 767; EIMS *m/z* (rel. int.): 502 $[M]^+$ (4), 406 (8), 388 (11), 342 (5), 198 (9), 137 (100), 109 (38), 95 (14); HREIMS *m/z* 502.1835 $[M]^+$ (calcd for $C_{26}H_{30}O_{10}$, 502.1839); for ^1H and ^{13}C NMR spectroscopic data, see Table 1.

4.7. Trijugins G and H (4–5)

Data on 1:1 equilibrium mixture. White amorphous powder; $[\alpha]_D^{20} - 17.0$ (*c* 0.120, MeOH); UV (MeOH), no maxima above 210 nm; IR (KBr) ν_{max} cm^{-1}: 3446, 2974, 1736, 1221, 1144, 1072, 1014, 983; EIMS *m/z* (rel. int.): 600 $[M]^+$ (19), 518 (14), 505 (20), 498 (6), 449 (28), 402 (15), 257 (24), 247 (30), 230 (50), 187 (19), 134 (64), 106 (69), 85 (80), 57 (100); HREIMS *m/z* 600.2574 $[M]^+$ (calcd for $C_{32}H_{40}O_{11}$, 600.2571); for ^1H and ^{13}C NMR spectroscopic data, see Table 2.

4.8. Methyl 8α-hydroxy-8,30-dihydroangolensate (6)

White amorphous powder; $[\alpha]_D^{20} + 50.0$ (*c* 0.105, MeOH); UV (MeOH) λ_{max} (log ε) nm: 210 (3.74, sh); IR (KBr) ν_{max} cm^{-1}: 3396, 2925, 1736, 1716, 1460, 1387, 1280, 1169, 1066, 1028; EIMS *m/z* (rel. int.): 488 $[M]^+$ (92), 374 (20), 359 (16), 237 (100), 205 (70), 177 (31), 149 (42), 121 (98), 95 (32); ESIMS (positive mode) *m/z* (rel. int.): 977 $[2M+H]^+$ (87), 999 $[2M+Na]^+$ (100); ESIMS (negative mode) *m/z* (rel. int.): 533 $[M+HCOO]^-$ (100); HREIMS *m/z* 488.2403 $[M]^+$ (calcd for $C_{27}H_{36}O_8$, 488.2410); for ^1H and ^{13}C NMR spectroscopic data, see Table 2.

4.9. Trichiconnarin A (7)

Yellowish gum; $[\alpha]_D^{20} + 231.6$ (*c* 0.380, MeOH); UV (MeOH) λ_{max} (log ε) nm: 211 (3.80), 233 (3.71); IR (KBr) ν_{max} cm^{-1}: 2918, 2850, 1728, 1651, 1504, 1454, 1246, 1199, 1163, 1105, 1024, 875, 806, 602; EIMS *m/z* (rel. int.): 232 $[M]^+$ (3), 136 (100), 121 (8), 108 (8), 80 (20); HREIMS *m/z* 232.0732 $[M]^+$ (calcd for $C_{13}H_{12}O_4$, 232.0736); for ^1H and ^{13}C NMR spectroscopic data, see Table 3.

4.10. Trichiconnarin B (8)

Yellowish gum; $[\alpha]_D^{20} + 87.0$ (*c* 0.105, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 214 (3.71, sh), 246 (3.49, sh), 309 (3.54); IR (KBr) ν_{max} cm^{-1}: 2924, 2852, 1728, 1620, 1458, 1383, 1228, 1163, 1026, 875, 602; EIMS *m/z* (rel. int.): 272 $[M]^+$ (3), 176 (100), 148 (36), 136 (13), 133 (22), 120 (16), 105 (40); HREIMS *m/z* 272.1048 $[M]^+$ (calcd for $C_{16}H_{16}O_4$, 272.1049); for ^1H and ^{13}C NMR spectroscopic data, see Table 3.

4.11. 3β,4α-dihydroxypregnan-16-one (9)

White amorphous powder; $[\alpha]_D^{20} + 67.0$ (*c* 0.105, MeOH); UV (MeOH), no maxima above 210 nm; IR (KBr) ν_{max} cm^{-1}: 3386, 2935, 2848, 1705, 1448, 1356, 1064, 1045, 592; EIMS *m/z* (rel. int.): 334 $[M]^+$ (94), 316 (100), 301 (28), 288 (70), 249 (36), 231 (42), 213 (43), 95 (60), 84 (85), 81 (56); HREIMS *m/z* 334.2514 $[M]^+$ (calcd for $C_{21}H_{34}O_3$, 334.2508); for ^1H and ^{13}C NMR spectroscopic data, see Table 4.

Table 4
^1H and ^{13}C NMR spectroscopic data for compound **9**[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
1α	1.70 (*m*)	36.2	12α	1.41 (*m*)	38.9
1β	1.06 (*m*)		12β	1.99 (*m*)	
2α	1.85 (*m*)	28.3	13		44.1
2β	1.51 (*m*)		14	1.11 (*m*)	56.6
3	3.34 (*m*)	76.4	15α	1.23 (*m*)	24.3
4	3.27 (*m*)	75.4	15β	1.65 (*m*)	
5	1.01 (*m*)	50.6	16α	1.64 (*m*)	22.8
6α	1.88 (*m*)	22.5	16β	2.14 (*m*)	
6β	1.16 (*m*)		17	2.50 (*m*)	63.8
7α	1.76 (*m*)	31.4	18	0.59 (*s*, 3H)	13.4
7β	0.87 (*m*)		19	1.83 (*s*, 3H)	13.6
8	1.35 (*m*)	34.9	20		209.7
9	0.73 (*m*)	54.2	21	2.10 (*s*, 3H)	31.5
10		37.2			
11α	1.60 (*m*)	20.9			
11β	1.29 (*m*)				

[a] Recorded in $CDCl_3$ at 400 and 100 MHz for ^1H and ^{13}C NMR, respectively. δ in ppm and *J* in Hz are in the parentheses.

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