A pentanortriterpenoid with a novel carbon skeleton and a new pregnane from *Trichilia connaroides*

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Abstract: A new rearranged pentanortriterpenoid (trijugin C) and a new pregnane (3β,4α-dihydroxypregnan-16-one) were isolated from the EtOH extract of *Trichilia connaroides* (Meliaceae). Trijugin C has been shown to be a derivative of methyl angolensate with a novel carbon skeleton. The structures were elucidated on the basis of spectral analysis including ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY experiments. The possible biogenetic pathway of trijugin C is discussed.

Key words: Trichilia connaroides, Meliaceae, pentanortriterpenoid, trijugin C, pregnane.

Résumé : On a isolé un nouveau pentanortriterpénoïde réarrangé, la trijucine C, et un nouveau dérivé du prégnane, la 3β,4α-dihydroxyprégnan-16-one, à partir des solutions éthanoliques d'extraction du *Trichilia connaroides* (Meliaceae). On a montré que la trijucine C est un dérivé de l'angolensate de méthyle à base d'un nouveau squelette carboné. On a élucidé les structures à l'aides d'analyses spectrales, y compris des expériences de « COSY », « HMQC », « HMBC » et « NEOSY » ^{1}H-^{1}H. On discute des voies de formations biogénétiques possibles de la trijucine C.

Mots clés : Trichillia connaroides, Meliaceae, pentanortriterpénoïde, trijucine C, prégnane.

[Traduit par la Rédaction]

Introduction

The roots of *Trichilia connaroides* Wight. & Arn. (syn: *Heynea trijuga* Roxb.) (Meliaceae) are used as a Chinese crude drug to treat arthritis, pharyngitis, tonsillitis, and other ailments (1). Four tetranortriterpenoids (trijugin A, trijugin B, trijugin B acetate, and 2-hydroxy-3-*O*-tigloyl-6-*O*-acetylswietenolide) have been isolated from the leaves and pericarps of this plant previously (2–4). Our investigation of the EtOH extract of the roots of *T. connaroides* resulted in the isolation of a rearranged pentanortriterpenoid (trijugin C (**1**)) with a new carbon skeleton, and a new pregnane (3β,4α-dihydroxypregnan-16-one (**2**)) (Fig. 1).

Results and discussion

Trijugin C (**1**) showed a $[M]^+$ ion at *m*/*z* 502.1837 in the HR-EI-MS spectrum, indicating a molecular formula of $C_{26}H_{30}O_{10}$. Twenty-six carbon signals were observed in the ^{13}C NMR spectrum. The multiplicities of the carbons determined by DEPT led to the attribution: 5 CH_3, 3 CH_2, 8 CH, 10 C, including one ketone (δ 217.2), three ester carbonyls (δ 174.9, 169.9, 168.9), a β-substituted furan (δ 143.2, 139.9, 121.5, 108.6), one methoxyl group (δ 52.9), three oxymethines (δ 80.8, 79.0, 74.1), and two oxyquaternary carbons (δ 92.3, 87.6). The absorption band at 3491 cm^{-1} in the IR spectrum showed the presence of the hydroxyl group. Based on the molecular formula and ^{13}C DEPT spectrum, only one hydroxyl was present in the structure. The ^{1}H and ^{13}C NMR spectra of **1** showed similarities to those of trijugin A (2). The signals of the β-substituted furan ring in the ^{1}H NMR spectrum occurred at δ 7.39 (H-21), 7.37 (H-23), and 6.25 (H-22) and the corresponding carbon signals at δ 139.9, 143.2 and 108.6, respectively, in the HMQC spectrum. Ring D was oxidized to a C-16 lactone, with H-17 oc-

Fig. 1. Structures of **1** and **2**.

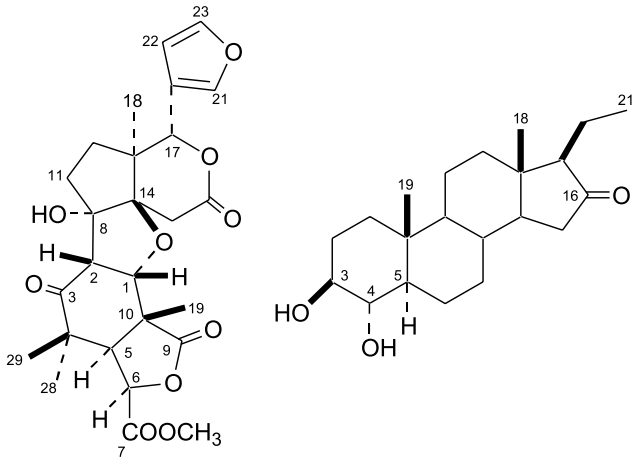

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Table 1. ^{1}H, ^{13}C, HMBC, and NOESY spectra data of trijugin C (**1**) (in $CDCl_3$).[a]

Position	δ_H	δ_C	HMBC correlation (H-C)	NOESY (H-H)
1	4.31 (d, 4.2)	79.0	C-3, 5, 10, 19	H-2, 11β, 12β, 19
2	2.81 (d, 4.2)	52.9	C-3, 8, 11, 14	H-1, 11β, 19, 29, OH
3		217.2		
4		47.9		
5	3.28 (d, 8.6)	45.4	C-1, 3, 4, 6, 9, 10, 19, 28, 29	H-6, 15β, 28
6	5.03 (d, 8.6)	74.1	C-5, 7, 9, 10	H-5, 28
7		169.9		
8		87.6		
9		174.9		
10		43.1		
11α	2.34 (m)	42.4	C-2, 8, 12	H-11β, 12α, 18
11β	2.07 (m)		C-2, 8, 12, 13, 14	H-1, 2, 11α
12α	1.50 (m)	33.0	C-11, 13, 17, 18	H-11α, 12β, 18
12β	1.73 (m)		C-8, 11, 13, 18	H-1, 12α
13		48.2		
14		92.3		
15α	2.88 (d, 17.0)	36.7	C-8, 14, 16	H-15β, 18
15β	2.09 (d, 17.0)		C-13, 14, 16	H-5, 15α
16		168.9		
17	5.62 (s)	80.8	C-12, 13, 14, 18, 20, 21, 22	H-12β
18	0.97 (s)	15.6	C-12, 13, 14, 17	H-11α, 12α, 15α, 22
19	1.56 (s)	19.1	C-1, 5, 9, 10	H-1, 2, 29
20		121.5		
21	7.39 (s)	139.9	C-22, 23	
22	6.25 (s)	108.6	C-20, 21, 23	
23	7.37 (s)	143.2	C-20, 21	
28	1.41 (s)	25.0	C-3, 4, 5, 29	H-5, 6, 29
29	1.11 (s)	20.9	C-3, 4, 5, 28	H-2, 19
OMe	3.85 (s)	52.9	C-7	
OH	5.87 (s)		C-2, 8, 11	H-2

[a]In ppm relative to the internal TMS run at 500 MHz. Multiplicities and splittings (Hz) are given in brackets.

Scheme 1. Proposed biogenetic pathway of trijugin C (**1**).

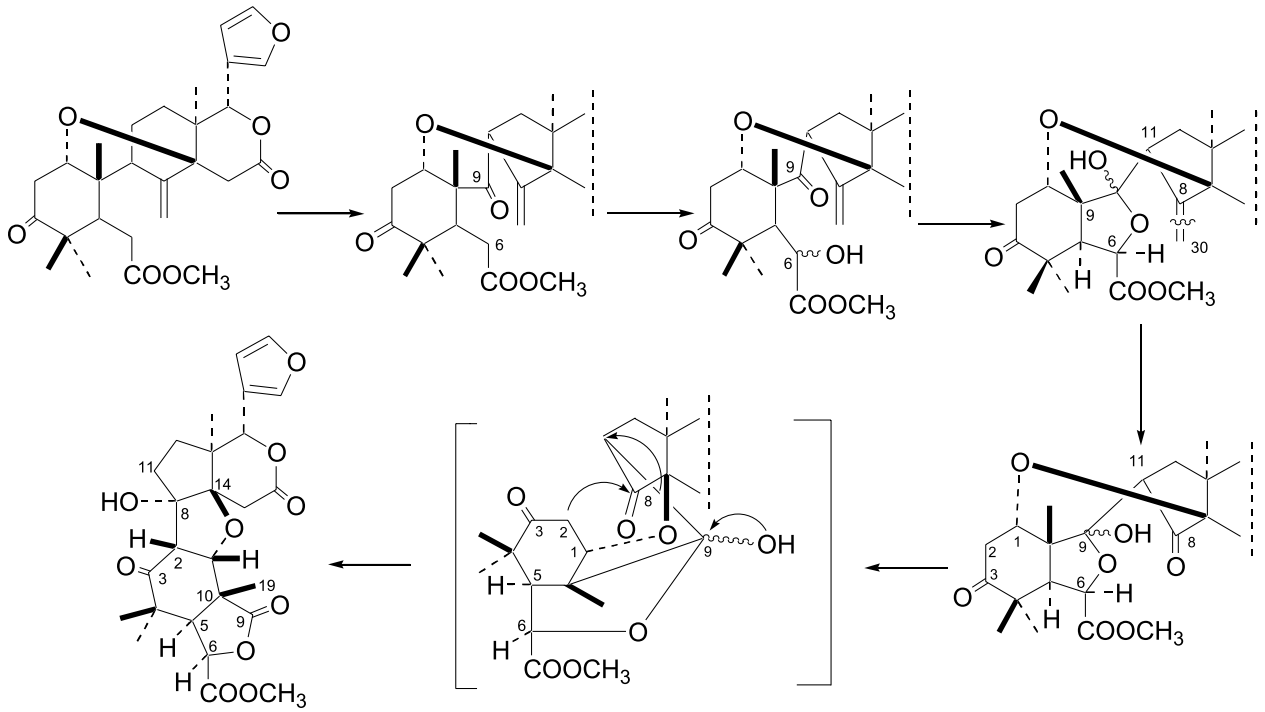
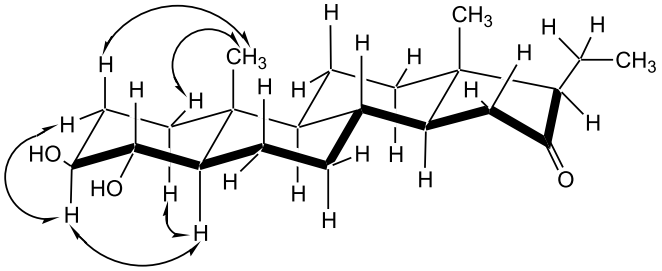


Fig. 2. Selected NOESY correlations of **2**.



curring as a singlet at δ 5.62 and H_2-15 occurring as a pair of doublets at δ 2.88 and 2.09 (J = 17.0 Hz). Four shielded methyl singlets at δ 1.56, 1.41, 1.11, and 0.97 were attributed to H_3-19, H_3-28, H_3-29, and H_3-18, respectively. The signal at δ 3.85 (3H, s) showed an HMBC correlation with an ester carbonyl carbon at δ 169.9 (Table 1), indicating the presence of a carbomethoxy group at C-7. H_2-11 (δ 2.34 (1H, m), 2.07 (1H, m)) and H_2-12 (δ 1.73 (1H, m), 1.50 (1H, m)) were assigned from their ^{1}H-^{1}H COSY correlations as well as an HMBC correlation to C-18. In addition, HMBC correlations were observed for H-15α (δ 2.88) to C-8 (δ 87.6) and C-14 (δ 92.3), and for H-11β (δ 2.07) to C-8 and C-14, supporting a contracted ring C formed by C-11, 12, 13, 14, 8. This was also confirmed by the absence of other coupling to the H_2-11 in the ^{1}H-^{1}H COSY spectrum.

In the HMBC spectrum, correlations were observed for H-6 (δ 5.03) to two ester carbonyl carbons C-7 (δ 169.9) and C-9 (δ 174.9), and for H_3-19 (δ 1.56) and H-5 (δ 3.28) to C-9. The presence of two C-11 protons and the absence of an HMBC correlation between H-11 and C-9 suggested that the C-9—11 bond had been cleaved. Thus, C-5, C-6, O, C-9, and C-10 formed a five-membered lactone ring.

The exchangeable sharp singlet at δ 5.87 (1H) in the ^{1}H NMR spectrum (removed on addition of D_2O) was ascribed to the proton of the hydroxyl group attached to C-8 supported by HMBC correlations between the hydroxyl proton and C-2 (δ 52.9), C-8 (δ 87.6), and C-11 (δ 42.4). Cross peaks observed from H-2 (δ 2.81) to C-3 (δ 217.2), C-8 (δ 87.6), C-11 (δ 42.4), and C-14 (δ 92.3), and from H_2-11 (δ 2.34, 2.07) to C-2, C-8, and C-14 suggested the linkage of ring A and ring C by the C-2—8 bond. Finally, a 1,14-ether linkage (as found in trijugin A) was proposed to satisfy the molecular formula and the deshielded nature of C-1 (δ 79.0) and C-14 (δ 92.3).

The relative stereochemistry at the chiral centers is suggested by the NOESY correlations summarized in Table 1. The NOESY plot showed a correlation between H_3-18 and H-22, suggesting the α-orientation of the furan ring at C-17, and correlations among H-2 and H-1, H-11β, H-12β, H_3-29 and H_3-19, revealing the β-orientation of H-2 and H-1 further supported by the small coupling constant (J = 4.2 Hz) of H-1 to H-2. Furthermore, the hydroxyl proton showed a correlation with H-2 but no correlation with H-1, H_2-11, and H_2-12, thus the hydroxyl group attached to C-8 was in the α orientation. Based on the above spectral data, the structure of trijugin C was determined to be as shown for **1**.

Usually tetranortriterpenoids with an open ring B have an 8,30 double bond. Trijugin class compounds have a rare contracted ring C and Connolly and co-workers (2) postulated that the trijugins may be formed by ring C contraction occurring by a pinacol–pinacolone rearrangement of a 9,11-dihydroxy precursor and anticipated more detailed examination of such compounds would be necessary. Up to now, 11 other analogues, E.P.4, E.P.5 (5), capensolactones 1, 2*a*, 2*b*, 3*a*, and 3*b* (6), voamatin A, B (7), and voamatin C, D (8) have been reported. These 14 compounds have either an 8,30 double bond or an 8,30-epoxide by oxidation, and either an exocyclic carbonyl or a hemi-ketal carbon atom at C-9. In pentanortriterpenoid **1**, C-30 has been removed by further oxidation of the 8,30 double bond. Retro-aldol cleavage of the C-9 (11) bond was followed by an aldol condensation between C-2 and C-8 to form a new ring. The cleavage and the formation of these C—C bonds have not been described before. A suggested biogenesis for **1**, starting with methyl angolensate (9), is given in Scheme 1. The oxidation of C-6 of tetranortriterpenoids with a lactone ring D and an opened ring B had been reported (10, 11).

Compound **2** had a molecular formula of $C_{21}H_{34}O_3$, as confirmed by HR-EI-MS (*m/z* 334.2511 $[M]^+$, calcd. 334.2508) and the ^{13}C NMR spectrum showing 21 carbon signals (3 C, 7 CH, 8 CH_2, 3 CH_3, including two oxymethines at δ 76.3, 75.3 and a ketonic carbonyl at δ 219.6). The absorption bands at 3564, 3271, and 1741 cm^{-1} in the IR spectrum were attributable to two hydroxyl groups and a five-membered ring ketone. The skeleton of **2** was similar to those of 2α,3β-dihydroxypregnan-16-one and 2β,3β-dihydroxypregnan-16-one (12, 13) by comparison of the NMR spectral data, as confirmed by ^{1}H-^{1}H COSY, HMQC, and HMBC spectra. The A/B ring junction was deduced as *trans* by the chemical shift value δ 13.5 of the C-19 methyl carbon (14). The two hydroxyl groups were concluded to be adjacent to each other, since the correlation was observed between the two oxymethine protons at δ 3.34 (1H, m) and 3.23 (1H, dd, J = 8.9, 11.2 Hz) in the ^{1}H-^{1}H COSY spectrum. Moreover, the correlations between the proton at δ 3.34 with a methylene group and the proton at δ 3.23 with a methine group indicated the only possibility for the positions of these two hydroxyl groups on the tetracyclic skeleton to be at C-3 and C-4. The large coupling constants (8.9 and 11.2 Hz) of H-4 indicated that H-3, H-4, and H-5 were axial. This result was also substantiated by H-5 and H-3, H-2α and H-3 showing spatial proximity in the NOESY experiment (Fig. 2). Thus, the structure of compound **2** was determined as 3β,4α-dihydroxypregnan-16-one.

Experimental

General procedure

The melting point was uncorrected. Instrumentation used for: $[\alpha]_D$: Horiba-300; NMR spectra: Bruker DRX-500; IR: Bio-Rab FTS-135; MS: VG Autospec-3000.

Plant material

The roots of *T. connaroides* were collected at Xishuangbanna, Yunnan Province, the People's Republic of China in August 1997 and identified by Professor Guoda Tao, Xishuangbanna Botany Garden, Chinese Academy of Sciences. A voucher specimen (No. 0620834) was deposited at the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Table 2. ^{1}H NMR, ^{13}C NMR, and HMBC spectra data of 3β,4α-dihydroxypregnan-16-one (**2**) (in $CDCl_3$).[a]

Position	δ_H	δ_C	HMBC correlation (H-C)
1α	1.67 (m)	35.9	C-2, 3, 5, 9, 10, 19
1β	1.04 (m)		C-2, 3, 9, 10, 19
2α	1.85 (m)	28.3	C-1, 3, 4
2β	1.49 (m)		C-1, 3, 4
3	3.34 (m)	76.3	C-2, 4
4	3.23 (dd, 8.9, 11.2)	75.3	C-3, 5, 6
5	1.01 (m)	50.6	C-4, 9, 10, 19
6α	1.91 (m)	22.4	C-5, 8, 10
6β	1.15 (m)		C-4, 5, 8, 10
7α	1.70 (m)	31.7	C-6, 9
7β	0.90 (m)		C-6, 9
8	1.49 (m)	34.0	C-7, 9, 10
9	0.85 (m)	54.4	C-11, 19
10		37.3	
11α	1.57 (m)	20.4	C-8, 9, 12, 13
11β	1.32 (m)		C-8, 9, 12, 13
12α	1.31 (m)	38.1	C-9, 11, 13, 18
12β	1.86 (m)		C-9, 11, 13, 18
13		42.0	
14	1.36 (m)	50.4	C-8, 9, 13, 15, 18
15	2.18 (dd, 10.8, 7.4)	38.4	C-13, 14, 16
	1.72 (dd, 10.8, 8.0)		C-8, 14, 16
16		219.6	
17	1.62 (m)	65.3	C-12, 13, 16, 18, 21
18	0.65 (s)	13.4	C-12, 13, 14, 17
19	0.85 (s)	13.5	C-1, 5, 9
20	1.60(m), 1.22 (m)	17.6	C-13, 16, 17, 21
21	0.99 (t, 7.4)	13.4	C-17, 20

[a]In ppm relative to the internal TMS run at 500 MHz. Multiplicities and splittings (Hz) are given in brackets.

Extraction and isolation

Dried and powdered roots (3.5 kg) of *T. connaroides* were extracted with 95% ethanol three times at room temperature. After removal of the solvent by evaporation, the residues (250 g) were suspended in H_2O, and then extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated to give a red extract (40 g), which was subjected to a Si gel column, eluting with a $CHCl_3$–Me_2CO mixture containing increasing amounts of Me_2CO to afford seven fractions. Fraction 6 was purified further on a Si gel column, eluted with $CHCl_3$–Me_2CO (4:1) to yield compound **1** (12 mg). Fraction 5 was rechromatographed on a Si gel column and eluted with $CHCl_3$–Me_2CO (10:1) to give compound **2** (32 mg).

Trijugin C (**1**): amorphous, mp 144–146 °C, $[\alpha]_D^{27}$ –49.4 (*c* 0.18, $CHCl_3$). IR (KBr) ν_{max} (cm^{-1}): 3491, 1743, 1636, 1503, 874. ^{1}H and ^{13}C NMR data, see Table 1. EI-MS *m/z* (%): 502 ($[M]^+$, 93) 487, 95 (100). HR-EI-MS *m/z*: 502.1837 $[M]^+$.

3β,4α-Dihydroxypregnan-16-one (**2**): colorless needles (petroleum ether – acetone), mp 152–154 °C, $[\alpha]_D^{27}$ –128.3 (*c* 0.31, $CHCl_3$). IR (KBr) ν_{max} (cm^{-1}): 3564, 3271, 1741, 1451, 1380, 1055, 1010. ^{1}H and ^{13}C NMR data, see Table 2. EI-MS *m/z* (%): 335 ($[M + H]^+$, 40), 334 ($[M]^+$, 82), 316 ($[M - H_2O]^+$, 13), 248 (100). HR-EI-MS *m/z*: 334.2511 $[M]^+$.

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