Tetrahedron Letters 50 (2009) 2132–2134

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Trichilin B, a novel limonoid with highly rearranged ring system from *Trichilia connaroides*

Zhao-Liang Geng[a,b], Xin Fang[a], Ying-Tong Di[a], Qiang Zhang[a], Ying Zeng[a], Yue-Mao Shen[a], Xiao-Jiang Hao[a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West of China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China
[b] Graduated School of Chinese Academy of Sciences, Beijing 100039, PR China

A R T I C L E I N F O

Article history:
Received 9 January 2009
Revised 18 February 2009
Accepted 20 February 2009
Available online 25 February 2009

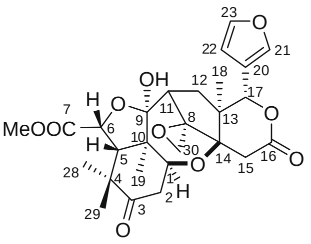
A B S T R A C T

Trichilin B (**2**), a limonoid having an unprecedented highly rearranged ring system, along with biosynthetically correlated trichilin A (**1**), was isolated from *Trichilia connaroides*. The structures were elucidated by spectroscopic analysis. Compound **2** features a unique 9,17-oxygen bridge, while two unprecedented δ-lactone rings fused to rings A and B at C-5, C-9 and ring C at C-8, C-14. Their cytotoxic activities were also evaluated.

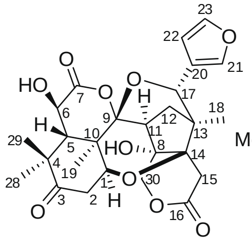
Limonoids from the Meliaceae family have attracted continuous attention due to their diverse structures and significant biological activites.[1] *Trichilia connaroides* is a treelet mainly distributed in India, Indonesia, and South of China, whose roots are used as primitive Chinese medicine for treating arthritis, pharyngitis, tonsillitis, and other ailments.[2,3] The extract of its bark also shows activities against lepidopteran larvae.[4] Previous phytochemical investigations on this species have led to 12 limonoids including five novel ring C contracted trijugin-type limonoids and four rearranged 30-nortrijugin-type limonoids, a mexicanolide-type, a methyl-angolensate-type limonoids, and two degraded limonoids.[5] As a continuing search for structurally novel limonoids from Meliaceae family, we investigated twigs and leaves of *T. connaroides* collected from Xishuangbanna, Yunnan Province of China, which led to a novel highly rearranged limonoid Trichilins B (**2**), together with biosynthetically correlated trichilin A (**1**). Trichilin B (**2**) has an unprecedented complex ring system featuring a unique 9,17-oxygen bridge, a 7,9-δ-lactone ring, and a 16,30-δ-lactone ring. Herein we describe the isolation and structural elucidation of these two compounds.

The air-dried powder of the twigs and leaves (4.8 kg) of *T. connaroides* was extracted with 95% EtOH at room temperature to give a crude extract, which was further extracted with petroleum ether, EtOAc, and n-BuOH, respectively. The EtOAc-soluble fraction was fractionated via an MCI gel column eluted with MeOH–H_2O (4:6 to 9:1) in gradient to obtain five fractions. Fraction 3 (5.5 g) was extensively chromatographed over columns of silica gel and C-18 reversed-phase silica gel, and finally purified by semipreparative HPLC to give trichilin A (**1**, 55 mg), and trichilin B (**2**, 5 mg).

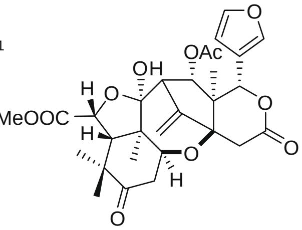
Trichilin A (**1**) was obtained as a white amorphous powder.[6] The molecular formula of $C_{27}H_{32}O_{10}$ was determined by the $[M+Na]^+$ ion peak at *m/z* 539.1896 (calcd 539.1893) in HRESIMS due to 12 degrees of unsaturation. The IR absorption bands showed the presence of hydroxyl group (3450 cm^{-1}) and ketone groups (1747, 1734, 1699 cm^{-1}). The 1D NMR spectra of **1** showed that two carbon-carbon double bonds and three carbonyls accounted for 5 out of 12 degrees of unsaturation, suggesting compound **1** to be heptacyclic. The ^{13}C spectrum (Table 1) showed the presence of 27 carbon signals. Apart from one carbon of methoxyl of methyl ester [δ_H



Trichilin A (**1**)



Trichilin B (**2**)



trijugin A (**3**)

---

* Corresponding author. Tel.: +86 871 5223263; fax: +86 871 5219684.
E-mail address: haoxj@mail.kib.ac.cn (X.-J. Hao).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.02.147

Table 1
^1H and ^{13}C NMR assignments of trichilin A (**1**) (in $CDCl_3$) and trichilin B (**2**) (in DMSO)

No.	**1** δ_H (multi, *J*, Hz)[a]	**1** δ_C[b]	**2** δ_H (multi, *J*, Hz)[a]	**2** δ_C[c]
1	4.62 (dd, 7.0, 3.0)	74.0	3.99 (m)	75.2
2α	2.95 (dd, 17.0, 7.0)	43.7 t	2.43 (dd, 11.0, 7.0)	41.2
2β	2.49 (dd, 17.0, 3.0)		3.47 (t, 11.0)	
3		211.6		213.6
4		46.6		46.0
5	3.18 (d, 8.0)	55.1	3.29 (d, 10.0)	43.4
6	4.80 (d, 8.0)	76.4	4.27 (dd, 10.0, 4.5)	69.3
7		175.6		173.9
8		64.1		79.6
9		110.0		101.8
10		54.2		41.4
11	2.73 (dd, 10.0, 4.5)	48.9	2.53 (d, 5.0)	36.9
12α	1.79 (dd, 13.5, 10.0)	35.7	1.93 (d, 13.5)	32.4
12β	2.20 (dd, 13.5, 4.5)		1.79 (dd, 13.5, 5.0)	
13		43.6		47.3
14		83.7		81.1
15a	2.58 (d, 17.8)	32.2	2.84 (d, 15.0)	38.5
15b	2.49 (d, 17.8)		2.26 (d, 15.0)	
16		168.2		171.2
17	5.88 (s)	79.7	4.83 (s)	77.2
18	0.90 (s)	17.2	0.89 (s)	15.8
19	1.27 (s)	17.8	0.89 (s)	18.5
20		121.4		124.1
21	7.42 (s)	139.8	7.51 (s)	140.9
22	6.29 (s)	108.1	6.54 (s)	111.6
23	7.40 (s)	143.6	7.70 (s)	141.6
28	1.10 (s)	20.0	1.09 (s)	18.7
29	1.30 (s)	27.8	1.22 (s)	30.2
30a	2.88 (d, 4.4)	51.4	4.07 (d, 13.0)	67.5
30b	2.83 (d, 4.4)		4.57 (d, 13.0)	
OMe	3.78 (s)	52.9		
6-OH			6.15 (d, 4.5)	
8-OH			3.7 (s)	

[a] Recorded at 500 MHz.
[b] Recorded at 100 MHz.
[c] Recorded at 125 MHz.

3.78 (s), δ_C 52.9], the remaining 26 carbons including a β-substituted furan ring [δ_H 7.42 (s, H-21), 6.29 (s, H-22), 7.40 (s, H-23); δ_C 121.4, 139.8, 108.1, 143.6] suggested a limonoid for compound **1**.[7]

An extensive comparison of ^1H and ^{13}C NMR data of trichilin A (**1**) with those of trijugin A (**3**),[5a] the limonoid isolated from the same species, shows they have nearly identical chemical shifts, which suggests they share the same skeleton. The only differences were the absence of the C-12 acetoxyl group and the 8,30 vinyl group being replaced by the 8,30 epoxide ring in **1**, which were supported by the absence of the signal of aforementioned group and by the presence of an additional methylene signal [δ_H 1.79 (dd, 13.5, 10.0), 2.20 (dd, 13.5, 4.5), δ_C 35.7] and a oxygenated methylene signal [δ_H 2.88 (d, 4.4), 2.83 (d, 4.4), δ_C 51.4]. HMBC correlations of the H_2-12/C-8, C-9, C-11, and Me-18, and H_2-30/C-8, C-11, and C-14 confirmed this proposition. A detailed 2D NMR analysis further established the planar structure of **1** as shown in Figure 1, suggesting **1** was derived from **3**. Thus a hydroxyl group was located in C-9 as supported by its chemical shift [δ 110.0] and the molecular formula of **1**, though no hydroxyl signal was observed in ^1H NMR spectrum. The relative configuration of **1** was assumed to be the same as those of **3** by the biogenetic point of view, therefore the configuration of 9-OH was assigned as α-oriented. The ROESY key correlations also confirmed the assigned relative configurations as shown in Figure 1.

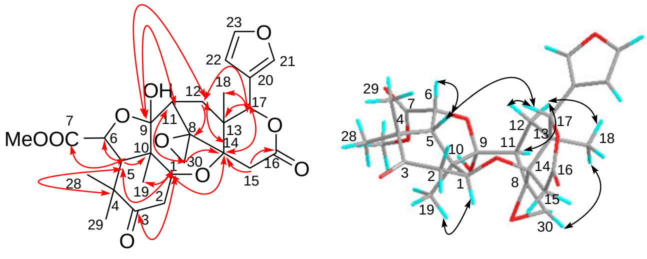


Figure 1. Selected HMBC (→) and key ROESY (↔) correlations of **1**.

Trichilin B (**2**), a white amorphous powder, showed the HREIMS ion at *m/z* 501.1765 $[M-H]^-$ corresponding to the molecular formula of $C_{26}H_{29}O_{10}$ (calcd 501.1760) with 12 degrees of unsaturation.[8] The IR spectrum showed absorption bands at 3436, 1753, and 1715 cm^{-1}, suggesting the presence of hydroxyl and carbonyls, respectively. The 1D NMR spectra revealed that 12 double-bond equivalents were occupied by two carbon-carbon double bonds and three carbonyls and thus indicated **2** to be heptacyclic. The ^{13}C spectrum (Table 1) resolved 26 carbon resonances including a β-furyl [δ_H 7.51 (s, H-21), 6.54 (s, H-22), 7.70 (s, H-23); δ_C 124.1, 140.9, 111.6, 141.6], suggesting a limonoid for compound **2**.

The ^1H and ^{13}C NMR data of **2** showed that the most significant feature of **2** was the absence of the methoxyl at C-7 characteristic of B-*seco*-type limonoid.[9] Taken together with the obviously upfielded ^{13}C chemical shifts of C-5, C-6, C-9, and C-10 compared with those of **1**, it should be a reasonable conclusion that the occurrence of hydrolysis of methyl ester of C-7 and the hemiketal (6,9-tetrahydrofuryl ring) was followed by the formation of 7,9-δ-lactone ring (ring-B). Although there was no direct HMBC correlation between C-7 and C-9, the observed 6-OH signal indicated by ^1H-^1H COSY correlation of 6-OH/H-6 and HMBC cross-peaks between 6-OH and C-5, C-6 strongly supported the hypothesis. The downfield ^{13}C signal of C-8 and C-30, and the assignment of 8-OH by HMBC correlation of 8-OH/C-8 suggested the break of 8, 30 epoxide ring. The absence of subsequent 30-OH proton and the HMBC cross signal of H_2-30/C-16 indicated the formation of 30, 16-δ-lactone ring. However, there was no corresponding proton resonance signal of 17-OH, and occurrence of typical upfield ^{13}C shifts of C-17 [δ 77.2] for ring-D hydrolyzed limonoid, implying C-17 be incorporated into a new ring.[10] Regarding to the disappearance of 9-OH signal, and the upfielded ^1H and ^{13}C signal of C-11 [δ_H 2.53 (d, 5.0), δ_C 36.9] and C-12 [δ_H 1.93 (d, 13.5), 1.79 (dd, 13.5, 5.0), δ_C 32.4], it was possible that a unique tetrahydropyranyl ring was formed between C-17 and C-9, which was confirmed by the HMBC correlation of H-17/C-9. Comprehensive analysis of 2D NMR data established the remaining structural fragments as shown in Figure 2, which were the same as those of **1**. The planar structure of **2** was thus figured out.

The relative configuration of **2** was achieved by analysis of its ROESY spectra (Fig. 2). The ROESY correlations of H-1/H-11 and H_3-19 indicated that H-1, H-11, and Me-19 were cofacial, arbitrarily assigned as the α-oriented. The ROESY cross-peaks from H-5 to H-2β and H_3-29 assigned the β-orientation of H-5 and Me-29, and accordingly the α-assignment of Me-28, in which H-

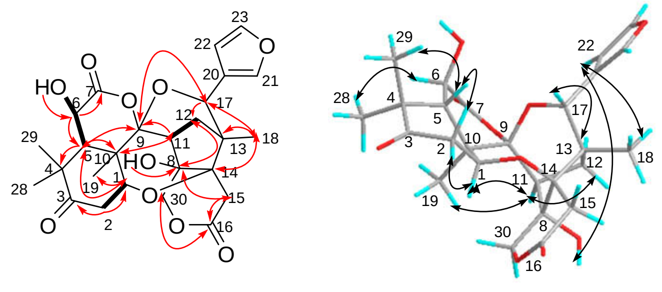
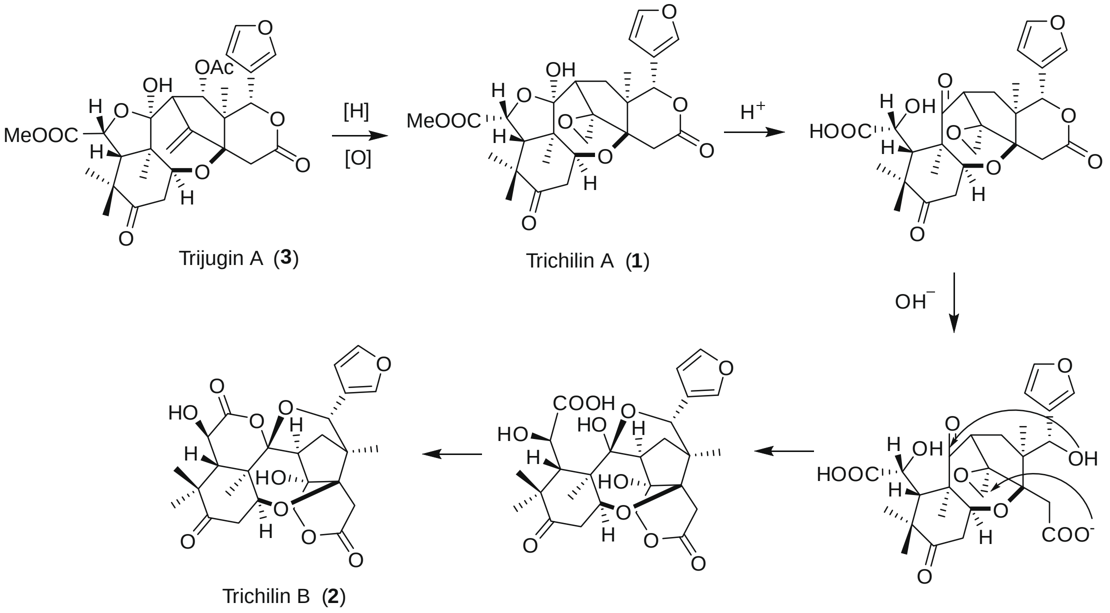


Figure 2. ^1H–^1H COSY (—) and selected HMBC (→) and key ROESY (↔) correlations of **2**.



Scheme 1. Hypothetical biosynthetic pathways of **1** and **2**.

2β was determined from H-2α as indicated by NOE cross-peak of H-1/H-2α. The orientation of H-6 was therefore assigned as α by NOE correlation of H-6/H_3-28, which was supported by the large coupling constants of $J_{5,6} = 10.0$ Hz. Similarly, the correlations of H-17/H-12β and H-11/H-12α suggested H-17 was β-oriented. Meanwhile, Me-18 and 8-OH were assigned as α-oriented by NOE cross-peaks of H_3-18 and 8-OH with H-22. This also allowed the cis configuration of ring E and ring D. Therefore, the structure of **2** was elucidated as shown in Figure 2.

A possible biosynthetic pathway from trijugin A (**3**) to trichilin A (**1**) and trichilin B (**2**) was proposed as shown in Scheme 1, which was supported by the nearly identical configurations of all chiral centers in **1**–**3**.

The experiment of the in vitro cytotoxicity of **1** and **2** against four tumor cell lines, HL-60, BEL7402, Hela, and MCF-7, were evaluated.[11] However, none of the compounds showed activity against the tumor cells (50% effective dose for clonal inhibition, $ED_{50} > 5$ μg/mL).

Acknowledgments

We thank Professor M.Y. Geng, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, for cytotoxicity testing. This work was financially supported by grants from the Ministry of Science and Technology (2009CB940900 and 2009CB522303).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.02.147.

References and notes

1. (a) Nakatani, M.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 1228–1230; (b) Tada, K.; Takido, M.; Kitanaka, S. *Phytochemistry* **1999**, *51*, 787–791; (c) Champagne, D. E.; Koul, O.; Isman, M. B.; Scudder, G. G. E.; Towers, G. H. N. *Phytochemistry* **1992**, *31*, 377–394; (d) Behenna, D. C.; Corey, E. J. *J. Am. Chem. Soc.* **2008**, *130*, 6720–6721; (e) Veitch, G. E.; Beckmann, E.; Burke, B. J.; Boyer, A.; Maslen, S. L.; Ley, S. V. *Angew. Chem., Int. Ed.* **2007**, *46*, 7629–7632.
2. Delectis Florae Reipubicae Popularis Sinicae Agendae Academiae Sinicae Edita, *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, China, 1997, *tomus 43 (3)*, p 66.
3. Jiangsu New Medical College *Dictionary of Chinese Crude Drugs*; Shanghai Scientific Technologic Press: Shanghai, China, 1977. p 1925.
4. Xie, Y. S.; Isman, M. B.; Gunning, P.; Mackinnon, S.; Arnasson, J. T.; Taylor, D. A.; Sanchez, P.; Hasbun, C.; Towers, G. H. N. *Biochem. Syst. Ecol.* **1994**, *22*, 129–136.
5. (a) Purushothaman, K. K.; Venkatanarasimhan, M.; Sarada, A.; Connolly, J. D.; Rycroft, D. S. *Can. J. Chem.* **1987**, *65*, 35–37; (b) Mathuram, V.; Kundu, A. B. *Indian J. Chem., Sect. B* **1990**, *29*, 970; (c). *J. Nat. Prod.* **1994**, *57*, 1446–1449; (d) Zhang, H. P.; Wu, S. H.; Shen, Y. M.; Ma, Y. B.; Wu, D. G.; Qi, S. H.; Luo, X. D. *Can. J. Chem.* **2003**, *81*, 253–257; (e) Wang, X. N.; Fan, C. Q.; Yin, S.; Gan, L. S.; Yue, J. M. *Phytochemistry* **2008**, *69*, 1319–1327.
6. Trichilin A (**1**): white amorphous powder; $[\alpha]_D^{25}$ −9.5 (*c* 0.63 $CHCl_3$); UV (MeOH) λ_{max} nm 204; IR (KBr) 3573, 3450, 1747, 1734, 1698, 1218, 1204, 1125, 1073, 1056, 1010 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m*/*z* 539.7 $[M+Na]^+$; HRESI-MS *m*/*z* 539.1896, (cacld for $[M+Na]^+$ 539.1893).
7. (a) Yin, S.; Wang, X. N.; Fan, C. Q.; Liao, S. G.; Yue, J. M. *Org. Lett.* **2007**, *9*, 2353–2356; (b) Wu, J.; Zhang, S.; Xiao, Q.; Li, Q. X.; Huang, J. S.; Long, L. J.; Huang, L. M. *Tetrahedron Lett.* **2004**, *45*, 591–593.
8. Trichilin B (**2**), white amorphous powder; $[\alpha]_D^{25}$ +25.6 (*c* 0.21 MeOH); UV (MeOH) λ_{max} nm 210; IR (KBr) 3436, 2923, 1753, 1715, 1611, 1123, 1110, 1080, 1048 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m*/*z* 501.5 $[M-H]^-$, 519.6 $[M+H_2O-H]^-$; HRESI-MS *m*/*z* 501.1765, (cacld for $[M-H]^-$ 501.1760).
9. (a) Saad, M. M. G.; Iwagawa, T.; Doe, M.; Nakatani, M. *Tetrahedron* **2003**, *59*, 8027–8033; (b) Fang, X.; Di, Y. T.; He, H. P.; Liu, H. Y.; Zhang, Z.; Ren, Y. L.; Gao, Z. L.; Gao, S.; Hao, X. J. *Org. Lett.* **2008**, *10*, 1905–1908.
10. (a) Chen, Y. Y.; Wang, X. N.; Fan, C. Q.; Yin, S.; Jian-Min, Yue *Tetrahedron Lett.* **2007**, *48*, 7480–7484; (b) Saad, M. M. G.; Iwagawa, T.; Doe, M.; Nakatani, M. *Tetrahedron* **2003**, *59*, 8027–8033.
11. (a) Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257–263; (b) Harada, N.; Uda, H. *J. Am. Chem. Soc.* **1978**, *100*, 8022.