

A New Limonoid from *Trichilia connaroides*

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A new limonoid, trichiliton B (**1**), was isolated from the twigs and leaves of *Trichilia connaroides*. Its structure was elucidated on the basis of spectroscopic analyses, including application of EI-MS, HR-ESI-MS, UV, IR, 1D and 2D NMR techniques.

Key words: *Trichilia connaroides*, Limonoid, Trichiliton B

Introduction

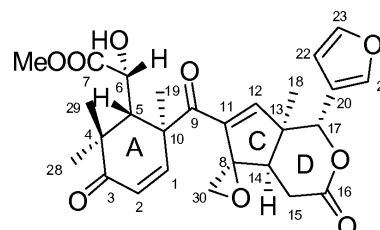
The plant *Trichilia connaroides* Wight & Arn (Meliaceae) is mainly distributed in India, Indonesia, and Southern China. Its roots are commonly used as primitive Chinese medicine. It is recorded in the Flora Reipublicae Popularis Sinicae and in the Dictionary of Chinese Crude Drugs for the treatment of arthritis, pharyngitis, tonsillitis, and other ailments [1,2]. Furthermore, the extract of its bark showed activities against lepidopteran larvae [3]. Limonoids, as the characteristic constituents of this genus, have attracted much attention for their structural diversity and significant biological activities. In previous work, five novel trijugin types, four rearranged 30-nontrijugin types, a mexicanolide type, a methyl angolensate type, and three degraded limonoids were isolated from *T. connaroides* [4–7]. In the continuing search for new limonoids, we have recently reported two novel limonoids from this species [8]. In this paper, we report the isolation and structure elucidation of a further new limonoid from the twigs and leaves of *T. connaroides* collected in Xishuangbanna, Yunnan Province of China.

Results and Discussion

Trichiliton B (**1**) was isolated as a colorless amorphous powder and showed a molecular formula $C_{27}H_{30}O_9$ by the $[M+Na]^+$ ion peak at $m/z = 521.1789$ (calcd. 521.1787) in HR-ESI-MS suggesting 13 degrees of unsaturation. Its IR absorption bands showed

Table 1. 1H (500 MHz) and ^{13}C (100 MHz) NMR data of trichiliton B (**1**).

no.	δ_H (mult, J in Hz)	δ_C	no.	δ_H (mult, J in Hz)	δ_C
1	6.47 (d, 10.0)	144.7	15 β	2.17 (dd, 12.8, 8.1)	41.7
			15 α	2.03 (dd, 12.8, 8.1)	
2	6.05 (d, 10.0)	128.5	16		168.7
3		201.9	17	5.42 (s)	81.4
4		44.5	18	1.17 (s)	19.2
5	3.17 (s)	47.1	19	1.45 (s)	21.0
6	4.31 (s)	70.7	20		120.1
7		175.8	21	7.53 (s)	140.5
8		61.9	22	6.39 (s)	108.9
9		208.1	23	7.46 (s)	143.7
10		54.1	28	1.03 (s)	26.6
11		163.9	29	1.19 (s)	22.8
12	5.73	108.7	30a	3.20 (d, 5.8)	57.2
13		44.0	30b	2.97 (d, 5.8)	
14	3.98 (t, 7.9)	45.9	OMe	3.78 (s)	53.1



Trichiliton B

the presence of a hydroxyl group (3438 cm^{-1}) and of carbonyl groups ($1724, 1681\text{ cm}^{-1}$). The 1D NMR spectra of **1** showed two carbon-carbon double bonds, three carbonyl groups and one β -substituted furan ring, which accounted for 8 out of 13 degrees of unsaturation, suggesting compound (**1**) to be pentacyclic.

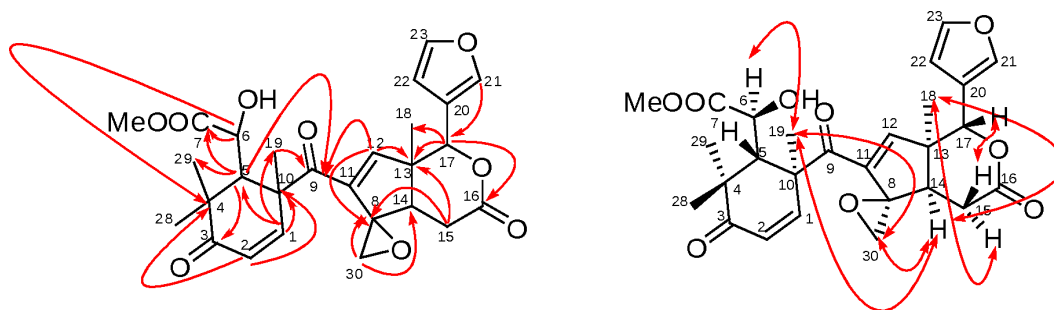


Fig. 1. Selected HMBC(→) and key ROESY (↔) correlations of **1** (color online).

The ^{13}C spectrum (Table 1) showed 27 carbon signals, which could be classified into 11 quaternary carbons (four carbonyls and two olefinic carbons), 9 methines (one oxygenated and six olefinic ones), 2 methylenes, and 5 methyls according to the DEPT spectrum. Apart from a methoxy group [$\delta_{\text{H}} = 3.78$ (s), $\delta_{\text{C}} = 53.1$], the remaining 26 carbons including a β -substituted furan ring [$\delta_{\text{H}} = 7.53$ (s, H-21), 6.39 (s, H-22), 7.46 (s, H-23); $\delta_{\text{C}} = 120.1, 140.5, 108.9, 143.7$] suggested a limonoid.

A comprehensive analysis of the 2D NMR spectra of **1**, including HMQC, HMBC, and ROESY experiments furnished its structure. The HMBC correlations of H-1 with C-5, C-10 and Me-19, and of H-5 with C-3 and H-2 with C-4, C-10 established a six-membered A ring with a C-6, C-7 side chain located at C-5 as suggested by HMBC correlations of H-5/C-7 and H-6/C-7. Though no corresponding proton signal was observed, the existence of a hydroxyl was supported by an IR absorption band at 3438 cm^{-1} . The hydroxyl group was finally located at C-6 by its chemical shift [$\delta_{\text{H}} = 4.31$ (s), $\delta_{\text{C}} = 70.0$]. Comparison of ^1H and ^{13}C NMR data of **1** with those of trichilin A [8] suggested that they share the same C, D ring system as well as the 8, 30 epoxide ring, which could be confirmed by HMBC correlations, as shown in Fig. 1. The rings A and C are connected through C-10–C-9–C-11 bonds as suggested by HMBC cross peaks of H₃-19 to C-9 and H-12 to C-9. Thus, the planar structure of **1** was established.

The relative configuration of **1** was deduced from the analysis of its ROESY correlations. As shown in Fig. 1, the observed correlations of H₃-19 with H-6, H₂-30, and H-14, as well as H₂-30 with H-14, H₃-18 indicated that H₃-19, H₃-18, H-6, H-14, and H₂-30 were at the same side in α -orientation, and thus a β -assignment for 6-OH results. The correlation of H-17 to H-15 β suggested that H-17 was β -oriented.

Although there was no ROESY correlation observed between H-5 and any other proton signal, the vicinal torsional angle between H-5 and H-6 appeared to be near 90° as suggested by the fact that both H-5 and H-6 displayed singlet signals in the ^1H spectrum, which indicated the β -orientation of H-5 [9]. Therefore, the structure of compound **1** was finally established as having a trijugin-type limonoid skeleton characterized by a five-membered ring C with an exocyclic carbonyl group at C-9 [5]. So far all reported trijugin-type limonoids have a 1,14 ether bridge for construction of the B ring of the compounds. However, as the first trijugin-type limonoid with an opened B ring, this 1,14 ether bridge is absent in trichiliton B (**1**).

The *in vitro* cytotoxicity of trichiliton B (**1**) against four tumor cell lines, HL-60, BEL7402, HeLa, and MCF-7, was studied [9, 10]. However, it showed no activity against the tumor cells (50% effective dose for clonal inhibition, $\text{ED}_{50} > 5\text{ }\mu\text{g mL}^{-1}$).

Experimental Section

General experimental procedures

One- and two-dimensional NMR experiments were performed on Bruker AM-400 MHz and DRX-500 MHz NMR spectrometers with tetramethylsilane as the internal standard. MS ((+)-ESI) and HRMS ((+)-ESI) spectral data were obtained on an API Qstar Pulsar I mass spectrometer. IR spectra were recorded on a Bio-Red FTS-135 spectrometer with KBr pellets. UV spectra were taken on a Shimadzu 2401 PC spectrophotometer. RP-18 silica gel (40–63 μm) and Sephadex LH-20 (40–70 μm) were bought from Merck, Germany and Amersham Pharmacia Biotech AB, Sweden, respectively. Silica gel (200–300 mesh) for column chromatography and GF254 were obtained from Qingdao Marine Chemical Inc., Qingdao, P.R. China. Thin-layer chromatography (TLC): silica gel GF-254 plates, detection at 254 nm UV light or by heating after spraying with 5% H_2SO_4 in EtOH (v/v). Compounds were detected under UV

light (254 nm) or by spraying with an anisaldehyde sulfuric acid solution followed by heating. Chemical shifts were reported using TMS as internal standard. Semi-preparative HPLC was performed on a Zorbax SB-C-18 (Agilent Co. Ltd. U. S. A.) column (i. d. 9.4×250 mm), developed with $\text{CH}_3\text{CN-H}_2\text{O}$ (45 : 55, 25 min) (flow rate, 2.0 mL min^{-1} ; detection, UV 210 nm) at 30°C .

Plant material

The twigs and leaves of *T. connaroides* were collected from Xishuangbanna, Yunnan Province, People's Republic of China, in May 2008. Its identity was confirmed by Mr. Jing-yun Cui (Xishuangbanna tropical botanical garden, CAS). A voucher specimen (no. Tc20080510) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming.

Extraction and isolation

The dried and milled twigs and leaves of *T. connaroides* (4.8 kg) were powdered and extracted with 95 % EtOH at r. t. to give a crude extract, which was further extracted with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble

fraction (28 g) was fractionated *via* a MCI gel column eluted with $\text{MeOH-H}_2\text{O}$ [from $\text{MeOH-H}_2\text{O}$ (4 : 6) to $\text{MeOH-H}_2\text{O}$ (9 : 1)] in gradient, to obtain five fractions (I–V). Fraction III (5.5 g) was extensively chromatographed over columns of silica gel (100 g) and C-18 reversed-phase silica gel, and finally purified by semipreparative HPLC to give trichiliton B (**1**, 6 mg).

Trichiliton B (**1**)

Colorless amorphous powder. – $[\alpha]_{\text{D}}^{25} = +117.6$ ($c = 0.17$, CHCl_3). – UV (MeOH) $\lambda_{\text{max}} = 204 \text{ nm}$. – IR (KBr): $\nu = 3438, 2923, 1724, 1680, 1460, 1242, 1024 \text{ cm}^{-1}$. – MS ((+)-ESI): $m/z = 499.2$ $[\text{M}+\text{H}]^+$. – HRMS ((+)-ESI): $m/z = 521.1798$ (calcd. 521.1787 for $\text{C}_{27}\text{H}_{30}\text{O}_9$, $[\text{M}+\text{Na}]^+$). – ^1H NMR (500 MHz, MeOH) and ^{13}C NMR (100 MHz, MeOH) spectral data: see Table 1.

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