A New Limonoid from *Trichilia connaroides*

Zhao-Liang Genga, Xin Fanga, Ying-Tong Dib, Qiang Zhanga, Yue-Mao Shen⁴, and Xiao-Jiang Haoa

a State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650204, P. R. China

b Guizhou Tobacco Research Institute, North Yuntan Road, Jinyang New District, Guiyang, 550023, P. R. China

Reprint requests to Prof. Dr. Xiao-Jiang Hao. Fax: +86-871-5150227. E-mail: haoxj@mail.kib.ac.cn

Z. Naturforsch. 2010, 65b, 1 – 3; received October 22, 2009

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 the presence of a hydroxyl group (3438 cm⁻¹) and of carbonyl groups (1724, 1681 cm⁻¹). The 1D NMR spectra of I 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.

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

<table>
<thead>
<tr>
<th>no.</th>
<th>δ_H (mult, J in Hz)</th>
<th>δ_C</th>
<th>no.</th>
<th>δ_H (mult, J in Hz)</th>
<th>δ_C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.47 (d, 10.0)</td>
<td>144.7</td>
<td>15β</td>
<td>2.17 (dd, 12.8, 8.1)</td>
<td>41.7</td>
</tr>
<tr>
<td>2</td>
<td>6.05 (d, 10.0)</td>
<td>128.5</td>
<td>15α</td>
<td>2.03 (dd, 12.8, 8.1)</td>
<td>168.7</td>
</tr>
<tr>
<td>3</td>
<td>2.17 (dd, 12.8, 8.1)</td>
<td>41.7</td>
<td>17</td>
<td>5.42 (s)</td>
<td>81.4</td>
</tr>
<tr>
<td>4</td>
<td>2.03 (dd, 12.8, 8.1)</td>
<td>168.7</td>
<td>18</td>
<td>1.17 (s)</td>
<td>21.0</td>
</tr>
<tr>
<td>5</td>
<td>3.17 (s)</td>
<td>47.1</td>
<td>19</td>
<td>1.45 (s)</td>
<td>21.0</td>
</tr>
<tr>
<td>6</td>
<td>4.31 (s)</td>
<td>70.7</td>
<td>20</td>
<td>1.03 (s)</td>
<td>26.6</td>
</tr>
<tr>
<td>7</td>
<td>175.8</td>
<td>21</td>
<td>140.5</td>
<td>21</td>
<td>7.53 (s)</td>
</tr>
<tr>
<td>8</td>
<td>61.9</td>
<td>22</td>
<td>108.9</td>
<td>23</td>
<td>6.39 (s)</td>
</tr>
<tr>
<td>9</td>
<td>208.1</td>
<td>23</td>
<td>143.7</td>
<td>28</td>
<td>1.03 (s)</td>
</tr>
<tr>
<td>10</td>
<td>54.1</td>
<td>28</td>
<td>143.7</td>
<td>29</td>
<td>1.19 (s)</td>
</tr>
<tr>
<td>11</td>
<td>163.9</td>
<td>29</td>
<td>57.2</td>
<td>30a</td>
<td>3.20 (d, 5.8)</td>
</tr>
<tr>
<td>12</td>
<td>3.73</td>
<td>30b</td>
<td>5.8</td>
<td>30b</td>
<td>2.97 (d, 5.8)</td>
</tr>
<tr>
<td>13</td>
<td>44.0</td>
<td>30b</td>
<td>53.1</td>
<td>30b</td>
<td>3.78 (s)</td>
</tr>
</tbody>
</table>

![Trichiliton B](image)
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 [$\delta_H = 3.78$ (s), $\delta_C = 53.1$], the remaining 26 carbons including a $\beta$-substituted furan ring [$\delta_H = 7.53$ (s, H-21), 6.39 (s, H-22), 7.46 (s, H-23); $\delta_C = 120.1, 140.5, 108.9, 143.7$] 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 [$\delta_H = 4.31$ (s), $\delta_C = 70.0$]. Comparison of $^1$H 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 H$_3$-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 H$_3$-19 with H-6, H$_2$-30, and H-14, as well as H$_2$-30 with H-14, H$_3$-18 indicated that H$_3$-19, H$_3$-18, H-6, H-14, and H$_2$-30 were at the same side in $\alpha$-orientation, and thus a $\beta$-assignment for 6-OH results. The correlation of H-17 to H-15$\beta$ suggested that H-17 was $\beta$-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 $^1$H spectrum, which indicated the $\beta$-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, ED$_{50}$ > 5 $\mu$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 Palsar 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 $\mu$m) and Sephadex LH-20 (40–70 $\mu$m) were bought from Merck, Germany and Amersham Pharmacia Biotech AB, Sweden, respectively. Silica gel (200–300 mesh) for column chromatography and GF254 was 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$_2$SO$_4$ 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 CH$_3$CN-H$_2$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 MeOH-H$_2$O [from MeOH-H$_2$O (4 : 6) to MeOH-H$_2$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. – $[\alpha]_{D}^{25} = +117.6$ (c = 0.17, CHCl$_3$). – UV (MeOH) $\lambda_{\text{max}} = 204$ nm. – IR (KBr): $\nu = 3438, 2923, 1724, 1680, 1460, 1242, 1024$ cm$^{-1}$. – MS ((+)-ESI): $m/z = 499.2$ [M+H]+. – HRMS ((+)-ESI): $m/z = 521.1798$ (calcld. 521.1787 for C$_{27}$H$_{30}$O$_{9}$, [M+Na]+). – $^1$H NMR (500 MHz, MeOH) and $^{13}$C NMR (100 MHz, MeOH) spectral data: see Table 1.

**Acknowledgements**

The authors express their gratitude to 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).

---