Short communication

Monoterpenoids and Triterpenoids from *Pterocephalus hookeri* with NF-κB inhibitory activity

Yingchun Wu, Jing Lu¹, XiaoQiuYu Lu, Ran Li, Jing Guo, Fujiang Guo*, Yiming Li*

School of Pharmacy, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, PR China

**A R T I C L E  I N F O**

Article history:
Received 30 January 2015
Received in revised form 6 May 2015
Accepted 13 May 2015
Available online 27 May 2015

Keywords:
*Pterocephalus hookeri*
Monoterpenoids
Triterpenoids
Inhibition of NF-κB

**A B S T R A C T**

In this study, we isolated two new monoterpenoids hookerinoids A and B (1 and 2; rare arranged nonglycosidic bis-iridoids) and hookerinoid C (3; a novel norursane-type triterpenoid) in addition to two known compounds, 11,12-epoxy-2,6-dihydroxy-24-norursa-1,4-dien-3-oxo-2-one (28→13)-olide (4) and rivularcin (5), from *Pterocephalus hookeri*. The structures of 1–3 were established using one-dimensional and two-dimensional nuclear magnetic resonance spectroscopy and high-resolution electrospray ionisation mass spectrometry. All compounds were isolated from this plant for the first time. Bis-iridoids isolated from *P. hookeri* possessed secoiridoid/iridoid subtype skeletons. Therefore, bis-iridoids can be considered chemotaxonomic markers of *P. hookeri*. The origins of the new compounds (1–3) were postulated and their inhibitory activities on a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway were assayed; 1 and 2 showed obvious activity in inhibiting NF-κB.

©2015 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.

1. Introduction

The genus *Pterocephalus*, a member of the family Dipsacaceae, comprises approximately 25 species distributed mainly in Europe and Asia and two species, *P. hookeri* (C. B. Clarke) Höek and *P. bretschneideri* (Batal.) E. Pritz, distributed mainly in China. *P. hookeri* is one of the most popular Tibetan herbs; it has been widely used in various Tibetan medicine prescriptions and has multiple traditional uses in treating illnesses such as cold, flu, rheumatoid arthritis, and enteritis (Gülçemal et al., 2010 Zhang et al., 2009). Several triterpenoids (Tian et al., 1993; Yang et al., 2007), iridoids (Tian et al., 2000), and bis-iridoids (Wu et al., 2014) have been isolated in previous chemical investigations. In this study, three new compounds (Fig. 1), two rare arranged nonglycosidic bis-iridoids (1 and 2) and one triterpenoid (3) along with two known triterpenoids, were isolated from the 95% EtOH extract of the underground parts of *P. hookeri*. Remarkably, in this study, all bis-iridoids isolated from *P. hookeri* exhibited secoiridoid/iridoid subtype skeletons comprising secoliganic acid that condensed to the 7-OH of loganin or loganin-like iridoids. Therefore, bis-iridoids can be considered chemotaxonomic markers of *P. hookeri*.

To determine bioactive natural products that are nuclear factor κB inhibitors, we also evaluated the inhibitory activity of new compounds 1–3 on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). NF-κB, which is a nuclear transcription factor, regulates the expression of numerous genes that are critical for regulating apoptosis, viral replication, tumorigenesis, and inflammation in addition to various autoimmune diseases. In addition, NF-κB is crucial for inflammatory response, and the aberrant activation of NF-κB is frequently observed in numerous cancers. Therefore, NF-κB signaling inhibitors were considered potential therapeutic agents for cancer and inflammatory diseases (Gilmore, 2006 Brasier et al., 2006; Karin et al., 2004). This paper presents the isolation and structure elucidation of new compounds (1–3) and their inhibitory activities for NF-κB.

2. Experimental

2.1. General experimental procedures

2.1.1. Plant material

Underground parts of *P. hookeri* were collected in August 2012 from Tibet, China, and were characterised by Prof. Zhi-Li Zhao (Shanghai University of Traditional Chinese Medicine). A voucher specimen (No. 20120817) was deposited at the School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, PR China.

*Corresponding authors. Tel.: +86 21 51322191; fax: +86 21 51322193.
E-mail addresses: gj@shuctmc.edu.cn (F. Guo); ymlius@163.com (Y. Li).
¹ Co-first author

http://dx.doi.org/10.1016/j.phytol.2015.05.012
1874-3900 © 2015 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
2.2. Extraction and isolation

Air-dried and powdered underground parts of *P. hookeri* (10 kg) were extracted using 95% EtOH (3 × 10.0 L) three times under reflux conditions. The EtOH extract was filtered and concentrated at a reduced pressure to yield a crude extract (1.2 kg). The extract was suspended in H₂O (4.0 L) and then successively partitioned using petroleum ether (3 × 4.0 L), CHCl₃ (3 × 4.0 L), and n-BuOH (3 × 4.0 L). The n-BuOH soluble fraction (120 g) was first subjected to D101 macroporous resin column chromatography (3000 g, 8 × 120 cm) and eluted using H₂O, 20% EtOH, 40% EtOH, 60% EtOH, and 80% EtOH successively to produce five polar parts. The 60% EtOH portion (50.5 g) was fractionated on a silica gel column (1000 g, 4 × 100 cm), eluted using EtOAc/MeOH/H₂O/HOAc (30:1:1–1:1:1, each with a volume of 500 mL), and then purified using Sephadex LH-20 column (30 g, 3 × 200 cm, MeOH); furthermore, the reversed-phase silica gel chromatography (100 g, 3 × 50 cm) involving eluting using MeOH/H₂O (1:4–19:1, each 100 mL) was repeated to produce compounds 3 (35 mg) and 4 (56 mg). The 20% EtOH portion (30.5 g) was fractionated on a silica gel column, eluted using EtOAc/MeOH/H₂O/EtOH (30:1:1–1:1:1:1, each 100 mL), and purified using Sephadex LH-20 column (30 g, 3 × 200 cm, MeOH); in addition, reversed-phase silica gel chromatography (100 g, 3 × 50 cm) involving eluting using MeOH/H₂O (1:4–19:1, each 100 mL) was repeated to produce compound 5 (88.3 mg).

2.2.1. Hookerinoid A (1)

Hookerinoid A was obtained as a white amorphous powder, and it was processed and evaluated using the following materials and instruments: [α]D₂⁰ = −81.0 (c = 0.514, MeOH); ultraviolet (UV) (MeOH) λmax (log ε): 238 nm (4.24); infrared (IR) (KBr): 3358,
Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>1(^a)</th>
<th>2(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\delta_h) (multi, (j = 1, f) in Hz)</td>
<td>(\delta_c)</td>
</tr>
<tr>
<td>A-1</td>
<td>4.29 (dq, (j = 11.4, 6.2) Hz)</td>
<td>71.4</td>
</tr>
<tr>
<td>A-3</td>
<td>7.60 (s)</td>
<td>156.5</td>
</tr>
<tr>
<td>A-4</td>
<td>110.7</td>
<td>110.7</td>
</tr>
<tr>
<td>A-5</td>
<td>3.06 (m)</td>
<td>271</td>
</tr>
<tr>
<td>A-6</td>
<td>2.17 (m), 1.35 (m)</td>
<td>33.8</td>
</tr>
<tr>
<td>A-7</td>
<td>5.16 (dd, (j = 9.8, 3.0) Hz)</td>
<td>90.4</td>
</tr>
<tr>
<td>A-8</td>
<td>4.85 (d, (j = 1.1) Hz)</td>
<td>95.9</td>
</tr>
<tr>
<td>A-9</td>
<td>1.61 (ddd, (j = 11.4, 4.0, 11.1) Hz)</td>
<td>42.5</td>
</tr>
<tr>
<td>A-10</td>
<td>1.43 (d, (j = 6.2) Hz)</td>
<td>19.0</td>
</tr>
<tr>
<td>A-11</td>
<td>168.3</td>
<td>168.3</td>
</tr>
<tr>
<td>A-12</td>
<td>3.58 (dr, (j = 12.4, 7.1) Hz)</td>
<td>63.9</td>
</tr>
<tr>
<td>A-13</td>
<td>3.38 (dt, (j = 12.4, 7.1) Hz)</td>
<td>3.38 (dt, (j = 12.4, 7.1) Hz)</td>
</tr>
<tr>
<td>A-14</td>
<td>1.27 (t, (j = 7.1) Hz)</td>
<td>15.4</td>
</tr>
<tr>
<td>B-1</td>
<td>4.92 (d, (j = 6.2) Hz)</td>
<td>97.0</td>
</tr>
<tr>
<td>B-3</td>
<td>7.49 (s)</td>
<td>154.0</td>
</tr>
<tr>
<td>B-4</td>
<td>111.7</td>
<td>111.7</td>
</tr>
<tr>
<td>B-5</td>
<td>3.10 (m)</td>
<td>33.8</td>
</tr>
<tr>
<td>B-6</td>
<td>2.34 (m), 1.65 (m)</td>
<td>40.9</td>
</tr>
<tr>
<td>B-7</td>
<td>5.24 (dr, (j = 6.8, 1.6) Hz)</td>
<td>77.8</td>
</tr>
<tr>
<td>B-8</td>
<td>2.17 (m)</td>
<td>41.9</td>
</tr>
<tr>
<td>B-9</td>
<td>1.87 (ddd, (j = 14.6, 8.6, 6.2) Hz)</td>
<td>48.5</td>
</tr>
<tr>
<td>B-10</td>
<td>1.10 (d, (j = 6.8) Hz)</td>
<td>14.4</td>
</tr>
<tr>
<td>B-11</td>
<td>169.7</td>
<td>175.0</td>
</tr>
<tr>
<td>B-12</td>
<td>3.72 (s)</td>
<td>51.7</td>
</tr>
</tbody>
</table>

\(^a\) Data were measured in CD3OD at 600 MHz (\(^1\)H) and 150 MHz (\(^13\)C). Chemical shifts (\(\delta\)) are in ppm being relative to TMS.

2963, 2938, 2873, 1701, 1632, 1438, 1366, 1262; high-resolution electrospray ionisation mass spectrometry (HRESIMS) (pos.) /m/z 467.1920 [M]+ (calcd for C32H28O10: 467.1917); \(^1\)H nuclear magnetic resonance (NMR) (CD3OD, 600 MHz), \(^13\)C NMR (CD3OD, 150 MHz) (Table 1).

2.2.2. Hookerinoid B (2)

Hookerinoid B was obtained as a colourless powder, and it was processed and evaluated using the following materials and instruments: \(\delta_{13C} = -27.9 \ (c = 0.022, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} \ (\text{log } e)\): 238 nm (4.28); IR (KBr): 3361, 2922, 2853, 1701, 1631, 1408, 1410, 1265, 1199; HRESIMS (pos.) /m/z 509.2158 [M]+Na\(^+\) (calcd for C32H32O11 Na: 509.2165); \(^1\)H NMR (CD3OD, 600 MHz), \(^13\)C NMR (CD3OD, 150 MHz) (Table 1).

2.2.3. Hookerinoid C (3)

Hookerinoid C was obtained as a colourless powder, and it was processed and evaluated using the following materials and instruments: \(\delta_{13C} = +22.4 \ (c = 0.5, \text{MeOH})\); UV (MeOH) \(\lambda_{\text{max}} \ (\text{log } e)\): 225 nm (2.26); IR (KBr): 3343, 2943, 2867, 2851, 1776, 1632, 1408, 1457, 1261; HRESIMS (pos.) /m/z 470.3020 [M]+ (calcd for C32H32O7: 470.3015); \(^1\)H NMR (CD3OD, 600 MHz), Table 2, \(^13\)C NMR (CD3OD, 150 MHz) (Table 2).

2.3. Cell culture and NF-\(\kappa\)B reporter luciferase assay

HEK293 cells were cultured in a high-glucose Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C in a CO2 incubator. A day before transfection, \(2 \times 10^5\) cells were plated in 1000 μL of a growth medium without antibiotics. The cells were cultured overnight, and the cell density was controlled at approximately 70–80% during transfection. The

plasmid pRL-SV40 (containing the renilla luciferase gene driven by an SV40 promoter) was co-transfected for normalisation. Diluted experimental and coreporter vectors (50:1) were added to 50 μL of Opti-MEM I reduced serum medium without serum and mixed gently. A FuGENE transfection reagent (Promega) was mixed gently before use, and an appropriate amount of this reagent was diluted in 50 μL of Opti-MEM I medium. After incubating for 5 min at room temperature, the diluted DNA and diluted FuGENE were mixed gently and then incubated for 20 min at room temperature. Next, 100 μL of this mixture was added to each well, and the components were mixed by gently rocking the plate back and forth. The cells were incubated at 37 °C in a CO2 incubator for 5 h and the medium was replaced with 10% FBS in a DMEM containing antibiotics. After the cells were incubated at 37 °C in a CO2 incubator for 3 h, compounds 1, 2, and 3 (10, 25, and 50 μM/L, respectively) were added to each well containing the cells and medium, and all components were mixed gently. The cells were incubated for 1 h and 10 ng/mL TNF-α was subsequently added to each well. After their incubation at 37 °C in a CO2 incubator for 16 h, the cells were collected and their intracellular luciferase activities were detected; this detection was performed using a Dual-Luciferase Reporter 1000 assay system (Promega).

3. Results and discussion

Two bis-iridoids 1 and 2 and three triterpenoids 3–5 resulted from the repeated column chromatography and semipreparative reversed-phase HPLC of the 95% EtOH extract of P. hookeri. Among these, the structures of the new compounds 1–3 were elucidated using various spectroscopic methods. The known compounds 4
and 5, which were isolated from this plant for the first time, were characterised as 11,12-epoxy-2,6-dihydroxy-24-norursa-14-dien-3-on-2-one (28→13)-olide (4) (Saito et al., 2012) and rivularkin (5) (Yang et al., 2007) by comparing their spectral data with those reported in previous studies.

Hookerinoid A (1) was obtained as a white amorphous powder, and its molecular formula (C_{22}H_{30}O_{10}) was deduced using HRESIMS (m/z 467.1917, [M+H]⁺). The IR spectrum indicated the presence of OH (3358 cm⁻¹) and conjugated C=O (1632 cm⁻¹, 1659 cm⁻¹). The UV spectrum showed a maximum at 238 nm. The 1H NMR spectrum demonstrated signals for two olefinic protons at δ 7.60 (1H, s) and 7.49 (1H, s); three hemiacetal protons at δ 5.16 (1H, dd, J=9.8, 3.0 Hz), 4.85 (1H, d, J=1.1 Hz), and 4.92 (1H, d, J=6.2 Hz); two methyl groups at δ 1.43 (3H, d, J=6.2 Hz) and 1.10 (3H, d, J=6.8 Hz); one methoxyl group at δ 3.72 (3H, s); one ethoxyl group at δ 3.58 (1H, dt, J=12.4, 7.1 Hz); 3.88 (1H, dt, J=12.4, 7.1 Hz); and 1.27 (3H, t, J=7.1 Hz). These signals indicate the presence of an iodirid unit (Unit B) and secoiridoid unit (Unit A) in the molecule. Unit B was easily assigned to a loganic-type iodirid (Kocsis et al., 1993) because of the presence of a methyl group at δ 1.10, olefinic signals at δH 7.50 as well as at δC 154.0 and 111.6, and carbonyl signals at δC 169.7 (13C NMR data), which were attributed to C-3, C-4, and C-11, respectively. The lack of other signals in the spectrum can be attributed to the presence of a sugar unit, indicating that 1 is a nonglycosidic bis-iridoid. In total, 23 carbon signals were observed in the 13C NMR spectrum of 1: 11 of these signals matched with those of the esterified loganin aglycone. Results of distortionless enhancement by polarisation transfer 135 (DEPT 135) experiments indicated that among the remaining 12 signals, one comprised two methylene carbons, two contained quaternary carbons, and eight comprised methyl or methine carbons. One of the quaternary carbon signals at δC 168.3 confirmed the presence of a carbonyl group. A detailed analysis of the NMR data indicated that the structure of Unit A was extremely close to that of an 8-ethoxyl-moroniside aglycone moiety (Gross et al., 1986). Remarkably, in the heteronuclear multiple bond correlation (HMBC) spectra (Fig. 2), the proton signal at δH 1.43 (H-10A) showed a long-range correlation with the carbon resonance at δC 156.5 (C-3A) and no correlation with the carbon resonance at δC 90.4 (C-7A). Furthermore, the signal at δH 5.15 (H-7A) showed a long-range correlation with the carbon resonance at δC 99.5 (C-8A), indicating that methyl (10A) was bonded to 1A and not 8A. Moreover, the signals at δ 3.58 and δ 3.88 (H-12A) showed long-range correlations with the carbon resonance at δC 100.9 (C-8A), indicating that the ethoxyl group (12A and 13A) was located at 8A. These results indicate that the structure of the secoiridoid moiety is rearranged. The two units were bonded by an ester linkage between the C-7 (OH) of Unit B and the carbonyl group (C-11A) of Unit A because the proton signal at δH 5.24 (H-7B) showed a long-range correlation with the carbon resonance at δC 168.3 (C-11A). Furthermore, the relative configuration of 1 was determined using Nuclear Overhauser effect spectroscopy (NOESY). For the isonicotinic part (unit B), the bicyclic fused ring system is rather rigid and can be easily analysed using NOE experiments and coupling constants.

The correlations between H-5B (δ 3.10) and H-9B (δ 1.87), H-5B and H-6B (δ 2.34), H-6B (δ 1.65) and H-7B (δ 5.24), H-7B and H-8B (δ 2.17), H-9B and CH₃-10B (δ 1.10) in the NOESY spectrum suggest that H-9B and H-5B, as well as the C-7B hydroxy and C-8B methyl groups have β configurations and a cis relationship. The two remaining proton sets (H-1B and H-9B and H-8B and H-9B) are in a trans relationship, therefore, C-1B hydroxy groups have β configurations. Regarding the secoiridoid moiety (Unit A), the bicyclic fused ring system was rather rigid and could be easily analysed using the NOE and coupling constants. According to the correlations between H-5A (δ 3.06) and H-9A (δ 1.61), H-5A and H-6A (δ 2.17), H-6A (δ 2.17) and H-7A (δ 5.16), and H-9A and H-8A (δ 4.85) indicated in the NOESY spectrum (Fig. 2), H-9A and H-5A had β configurations and a cis relationship, whereas the C-7A hydroxy and C-8A ethoxyl groups had α configurations. The α configuration of the C-7A hydroxy group was determined by comparing the chemical shifts and spin coupling at δ 5.16 (dd, J=9.8, 3.0 Hz) with those of the 7-α-hydroxy morroniside (Gross and Sticher, 1986). According to the values of J₁₂₃₄₅ (11.4 Hz) and J₁₈₉,₁₀₀ (11.1 Hz) (Lin et al., 2011), H-1A and H-9A had a trans relationship, whereas H-8A and H-9A had a cis relationship. Therefore, the gross structure of 1 could be assigned to hookerinoid A (Fig. 1).

Hookerinoid B (2) was obtained as a white amorphous powder, and its molecular formula (C_{22}H_{30}O_{11}) was deduced using HRESIMS (m/z 509.2165 [M+Na]⁺). The IR spectrum indicated the presence of OH (3361 cm⁻¹), ester C=O (1701 cm⁻¹), and conjugated C=O (1631 cm⁻¹). The 1H and 13C NMR Tables 1 and 2 spectroscopic data of 1 and 2 were compared and the results indicated that 2 was closely related to 1 structurally. In the 1H and 13C NMR of 2, several signals were highly similar to those of 1; the only difference was that the olefinic constituents (3B and 4B) of Unit B were replaced by a hydroxyl group. This finding was supported by MS data, in which later showed 18 Da more. The NOE correlations between H-8B and H-7B, H-10B and H-9B, and H-1B, and no correlation between H-8B and H-9B confirmed that both the hydroxyl group at C-7B and methyl group at C-8B were β-oriented. The configurations at C-3B and C-4B were deduced from coupling constants; J₃₄₅B (7.2 Hz) and J₃₈₉₅B (10.2 Hz) showed that the configuration of the methoxycarbonyl group at 4B was β-equatorial and that of the hydroxy group at 3B was α-equatorial (Kocsis et al., 1993). The connectivity of 1H and 13C NMR signals was determined using heteronuclear multiple quantum coherence (HMQC), and the skeleton of 2 was further confirmed using HMBC and NOESY. Therefore, we concluded that the structure of 2 was hookerinoid B (Fig. 1).

Hookerinoid C (3) was obtained as a white powder, and its molecular formula (C_{22}H_{30}O_{12}) was deduced using HRESIMS (m/z 470.3015 [M+H]⁺). The IR spectrum indicated the presence of OH (3343 cm⁻¹) and ester C=O (1670 cm⁻¹). The 1H NMR spectrum

Fig. 2. Selected 2D NMR correlations for compound 1–3.
and aqueous extracts both demonstrate central and peripheral analgesic activities and anti-inflammatory effects (Zhang et al., 2009). However, reports on the chemical constituents of this plant are limited. We previously reported the bis-iridoids which found in P. hookeri have the inhibitory effect on NF-κB signaling (Wu et al., 2014). This time the new bis-iridoids result obtained in this study further indicates that bis-iridoids inhibit TNF-induced NF-κB activation, suggesting that this bis-iridoid is probably related to the anti-inflammatory effects of P. hookeri.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research program was supported by the program for Professors of Special Appointment (Eastern Scholar) in Shanghai Institutions of Higher Learning (for Y M Li), and the National Natural Science Foundation of China (No. 81173518).

References


