Research Article

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Two new polypodane-type bicyclic triterpenoids from mastic

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Abstract: Pistacia lentiscus L. is an evergreen shrub belonging to the Anacardiaceae family, cultivated exclusively in the southern area of Chios Island. Mastic gum as a unique natural resin of the tree Pistacia lentiscus L. has been used extensively in functional foods and traditional medicine. The biological properties of Pistacia resins may be influenced by different chemical constituents. Herein the present work is aimed to further explore the diverse triterpenoids of mastic, and evaluate their anti-inflammatory activity. Two undescribed polypodane-type bicyclic triterpenoids were isolated from the Pistacia resins, their structures were elucidated using ultraviolet, infrared, high resolution electrospray ionization mass spectroscopy (HRESIMS), and nuclear magnetic resonance data. LPS-stimulated RAW266.7 macrophages were incubated with various concentrations of isolated compounds, and results showed that compounds 1 and 2 inhibited nitric oxide production in LPS-induced RAW266.7 cells with IC50 values of 28.1 and 32.6 µM, respectively.

Keywords: Pistacia lentiscus, mastic, polypodane-type bicyclic triterpenoids, anti-inflammation

1 Introduction

Terpenes are a group of natural compounds which act on many steps of pathophysiological processes, and their chemical diversity is well suited to provide skeleton for anti-inflammatory remedies [1]. Several terpenes’ natural and synthetic derivatives showed anti-inflammatory activities in vitro and in vivo via inhibiting pro-inflammatory cytokine production, such as nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), prostaglandin E2 (PGE2), and interleukin-6 (IL-6) [2].

Mastic is a globular, pale yellow, resinous exudate of the evergreen shrub Pistacia lentiscus L. of the Anacardiaceae family, grown only in southern part of the island of Chios [3]. It has been widely used both as a food supplement and as a traditional medicine in the Mediterranean and Middle Eastern countries [4,5]. Clinically, mastic has been effective in the treatment of gastrointestinal disorders with less side effects [6–8]. Up to now, various beneficial pharmaceutical properties have been found in mastic, including antimicrobial, anti-inflammatory, anti-ulcer, antioxidant, antitumor, etc. [3,5]. Because of those wide spectrum of biological activities, which are mainly attributed to triterpenes and volatile compounds, mastic has been rediscovered and attracted much attention [3]. More than 120 chemical compounds, including some identified by GC-MS, were reported so far from the 1980s. The gum resins contain triterpenes (constituting 65–70%) comprising tetracyclic and pentacyclic triterpenes which are derivatives of tirucallane, dammarane, oleanane, and lupane skeletons [9], essential oil containing the major components α-pinene and β-myrcene [10], and a range of dicyclic triterpenes (1–2% of the total resin) [11]. Multiple lines of evidence suggest that Pistacia resins can inhibit the production of both NO and PGE2 in lipopolysaccharide (LPS)-activated RAW266.7 cells [12], decrease the IL-6, and C-reactive protein (CRP) plasma levels [13], and limit an increase in plasma free amino acids for maintenance and recovery of intestinal functions [14].

Our preliminary experiments revealed that terpenoids were abundant in mastic [15], and the crude extract of mastic could improve disease active index and colonic pathological changes in dextran sulfate sodium salt-induced ulcerative colitis mice. To further explore the chemical diversity of triterpenoids, two new polypodane-type bicyclic triterpenoids were isolated and identified.
In addition, their inhibitory effects against NO production in LPS-induced RAW264.7 cells were assayed.

## 2 Materials and methods

### 2.1 General experimental procedures

The equipment used in extraction and isolation of compounds include semi-preparative HPLC equipped with 5110 pump coupled to a 5430 diode array detector, a 5210 autosampler (Hitachi Limited, Tokyo, Japan), and Chiralpak AD-H (5 μm, 10 mm × 250 mm, Daicel, Tokyo, Japan). Instruments for structural characterization include Nuclear magnetic resonance Avance II 400 MHz (Bruker Co., Germany), Autopol IV automatic polarimeter (Rudolph Research Analytical, USA), Q-Exactive tandem mass spectrometer (Thermo Fisher Scientific, Waltham, USA), and JASCO J-720W spectrophotometer (Jasco, Tokyo, Japan). All reagents (HPLC or analytical-grade) were bought from Sigma-Aldrich and Tansoole (Shanghai, People’s Republic of China).

### 2.2 Plant material

*Pistacia* resins were purchased from Xinjiang Uygur Medicine Hospital (People’s Republic of China) and identified by Prof. Yan Wei, Xinjiang Agricultural University. A voucher specimen (MG-201810) was deposited in the University and College Key Lab of Natural Product Chemistry and Application in Xinjiang, Yili Normal University.

### 2.3 Extraction and isolation

Extraction and isolation were carried out according to the method previously described [15]. Briefly, a quantity of a crude methanol extract (175 g) was extracted successively with petroleum ether, ethyl acetate, and n-butanol. An MCI gel column was eluted with a gradient of MeOH/H2O (60:40–100:0) to separate the ethyl acetate extract into nine fractions (Fr. A–Fr. I). Fr. E (28.0 g) was subjected to column chromatography (CC) over ODS eluting with MeOH/H2O (50:50–100:0) to give eight subfractions (Frs. E1–E8). Frs. E4 (2.3 g) was chromatographed on silica gel CC, eluted successively with CH2Cl2/MeOH (50:1–0:1) to obtain subfraction E4b (523.2 mg). Following the purification by Sephadex LH-20 CC using MeOH, subfraction E4b3 (23.3 mg) were obtained and further separated by semi-preparative RP-HPLC (78:22, MeOH/H2O, 3.0 mL/min) to yield E4b3a (12.6 mg). Subfraction E4b3a via chiral-phase HPLC (AD-H column, n-hexane/isopropanol, 81:19; 3.0 mL/min) afforded 1 (3.7 mg, tR = 12.82 min) and 2 (3.8 mg, tR = 14.05 min).

#### 2.3.1 3β,8β,21-Trihydroxypolypoda-22-methoxy-13(1)(E),17(1)-diene (1)

Colorless amorphous solid; [α]D25 + 20° (c 0.1, MeOH); UV (MeOH) λmax 204 nm; IR νmax 3,440, 2,953, 2,849, 2,353, 2,324, 1,635, and 1,456 cm⁻¹; 1H (400 MHz) and 13C (100 MHz) NMR spectral data in CDCl3; Table 1; HRESIMS: m/z 515.4056 [M + Na]⁺ (calcd for C31H56O4Na, 515.4076).

#### Table 1: 1H NMR (400 MHz) and 13C NMR (100 MHz) data of 1 (in CDCl3)

<table>
<thead>
<tr>
<th>No.</th>
<th>δH (mult, J, Hz)</th>
<th>δC</th>
<th>No.</th>
<th>δH (mult, J, Hz)</th>
<th>δC</th>
<th>No.</th>
<th>δH (mult, J, Hz)</th>
<th>δC</th>
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<td>1.71 m, 1.17 m</td>
<td>38.0</td>
<td>12</td>
<td>2.06 m 2H</td>
<td>31.4</td>
<td>23</td>
<td>0.76 s 3H</td>
<td>15.5</td>
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<td>2</td>
<td>1.65 m, 1.59 m</td>
<td>27.3</td>
<td>13</td>
<td>5.17 t (7.1)</td>
<td>125.1</td>
<td>24</td>
<td>0.99 s 3H</td>
<td>19.1</td>
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<tr>
<td>3</td>
<td>3.25 dd (11.2, 4.8)</td>
<td>79.0</td>
<td>14</td>
<td></td>
<td></td>
<td>25</td>
<td>0.80 s 3H</td>
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<tr>
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<td>16</td>
<td>2.00 m 2H</td>
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<td>21</td>
<td>3.42 dd (10.2, 2.0)</td>
<td>76.4</td>
<td>22</td>
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</tbody>
</table>

Bold font represents carbon atom serial number.
2.4 Cytotoxicity assay

The RAW264.7 cell line purchased from the American Type Culture Collection (ATCC) were cultured in DMEM with 10% FBS. The cell viability assays of the isolated triterpenoids to RAW264.7 cells were evaluated by cell counting kit 8 (CCK-8, Dijindo, Japan) as described previously [16]. The cells at a density of 1.5 × 10^6 cells/well were seeded in 96-well microplates with 100 μL of DMEM containing 10% FBS and 1% penicillin-streptomycin in 5% CO2 at 37°C for 24 h. The optical density (OD) at 450 nm was measured using a microplate reader, and IC50 values were calculated by Graphpad Prism software (version 8.0).

2.5 NO assay

Detection of accumulated nitrites was performed using Griess reagent (Beyotime, Shanghai, China) as described previously [17]. Briefly, the RAW264.7 cells at approximately 1.5 × 10^5 cells/well were incubated for another 24 h. After that, the supernatant was removed, and CCK8 (10%, 100 μL) reagent was added into each well and incubated at 37°C for 2 h. The optical density (OD) at 540 nm was measured using a microplate reader, and IC50 values were calculated by Graphpad Prism software (version 8.0).

2.6 Statistical analysis

Data are expressed as mean value ± SD and analyzed by Graphpad Prism software (version 8.0).

3 Results and discussion

Compound 1 was isolated as a colorless oil with positive optical rotation (20, c 0.1, MeOH). Its molecular formula was determined as C_{31}H_{56}O_{12}Na by HRESIMS at m/z 515.4956 [M + Na]^+, together with its 13C NMR (Table 1) data, suggesting 4 degrees of unsaturation. The 1H NMR spectrum (Table 1) of 1 exhibited 8 methyl groups at δH 1.14, 1.12, 1.10, 0.99, 0.80, 0.76, each 3H, s; δH 1.61, 6H, s, two olefinic protons at δH 5.17 (2H, t, J = 7.1 Hz), two oxygenated methines at δH 3.42 (dd, J = 10.2, 2.0 Hz) and 3.24 (dd, J = 11.2, 4.8 Hz), and a methoxy at δH 3.22 (3H, s). The 13C NMR and HSQC spectra displayed the presence of 31 carbons comprising eight methyls, ten methylenes, six methines (including three oxygenated and two sp2 carbons), and six quaternary carbons (including two oxygenated and two sp2 carbons), and one methoxy. The abovementioned data together with literature research [18,19] implied that 1 is likely a polydopane-type bicyclic triterpenoid.

Further analysis of 1 using HSQC and H-1H COSY data revealed that compound 1 shared the same A and B rings with those of (8R)-3β,8-dihydroxypolypoda-13E,17E,21-triene [20]. The notable difference was that the double bond Δ21 group signal observed in (8R)-3β,8-dihydroxypolypoda-13E,17E,21-triene was absent in 1, while the presence of an additional methoxy at δH 3.22 (3H, s), δC 49.2, and one oxygenated methine at δH 3.42 (dd, J = 10.2, 2.0 Hz), δC 76.4, which were confirmed by the 1H-1H COSY correlations (Figure 1) between H-21 and H-19, H-20, and HMBC correlations (Figure 1) from H-29/H-30 to C-21 (δC 76.4), and C-22 (δC 77.6), and methoxy proton signal (δH 3.22) to C-22. The large coupling constant of H-3 (dd, J = 11.2, 4.8 Hz) suggested that 3-OH was β-oriented [21], and the NOESY correlation from H-3 to H-5, and H-24 supported this assignment. Therefore, the structure of 1 was proposed as 3β,8β,21-trihydroxypolypoda-22-methoxy-13(E),17(E)-diene.
We attempted to determine the absolute configurations of 1 using X-ray diffraction, circular dichroism (CD), and mosher’s method, but unfortunately all attempts have failed. In addition, compound 2 shows very similar data to 1, such as 1H NMR and 13C NMR spectra, HRESIMS and CD data. The most obvious difference was that compound 1 gave positive optical rotation, while compound 2 gave negative optical rotation. As the absolute configurations of rings A and B of natural polypodane-type bicyclic triterpenoid are confirmatory, the difference between 1 and 2 is speculated to stereo-isomerization at C-21. However, the absolute configuration of two compounds still needs further study.

The cell viability of RAW264.7 was kept more than 80% with the concentration of compounds and positive control dexamethasone from 10 to 40 µM (Figure 2), which excludes false positive results caused by the cytotoxicity. As shown in Figure 2, compounds 1 and 2 exhibited concentration-dependent manner at 10 to 40 µM, and they showed moderate inhibitory abilities against the production of NO with IC50 values at 28.1 and 32.6 µM, for comparison, dexamethasone had an IC50 value of 19.7 µM. Considering the anti-inflammatory effects previously reported for the source material [12,15,22], the isolated compounds were evaluated for inhibitory activity against NO production in LPS-induced RAW264.7 cells. The result showed that the isolated compounds possess potential inhibitory activity, which were comparable to that of positive control dexamethasone. Inhibition of NO production via MAPKs and NF-κB signaling pathways is a key anti-inflammatory approach. Bicyclic triterpenoids present in mastic contribute to the anti-inflammatory activity, but the potential mechanism of action was unclear and further research would be required to confirm this.

4 Conclusion

Inflammation is a defensive response to damaging factors in the host. While moderate inflammation exerts health beneficial effects, excessive produced pro-inflammatory cytokines will cause some adverse reactions. Mastic gum, which act as a traditional herbal medicinal product with good therapeutic effect on inflammation, is a complex mixture comprising different bioactive compounds in which each chemical constituent contributes to their overall bioactivity. Numerical results obtained from this research work have indicated two new polypodane-type bicyclic triterpenoids exhibited moderate inhibitory effects on anti-inflammatory activities in vitro through against NO release by LPS-stimulated RAW264.7 macrophages. These findings provide a foundation for illustrating the anti-inflammatory components, but underlying mechanisms still require further study.

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data analysis; Yu XH proofread the manuscript; Wu H carried out the biological tests; Liu W supervised the phytochemical experiments and revised the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All data supporting the findings of this study are available within the article.

References


