Fitoterapia 121 (2017) 159–163

Contents lists available at ScienceDirect

Fitoterapia

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

Triterpenoids from the bark of *Dysoxylum hainanense* and their anti-inflammatory and radical scavenging activity

Yu-Hai Zou[a,1], Wen-Ting Liu[b,1], Jin-Xia Zhang[a], Ding-Cheng Xiang[a,*]

[a] Department of Cardiology, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou 510010, China
[b] Department of Ent, Guangzhou First People's Hospital, Guangzhou 510180, China

ARTICLE INFO

Keywords:
Dysoxylum hainanense
Meliaceae
Triterpenoids
Anti-inflammatory
Radical scavenging activities

ABSTRACT

A phytochemical investigation on the 70% EtOH extract of the bark of *Dysoxylum hainanense* resulted in the isolation of four new triterpenoids, dysoxyhaines A–D (**1–4**). Structural elucidation of all the compounds were performed by spectral methods such as 1D and 2D (^{1}H–^{1}H COSY, HMQC, and HMBC) NMR spectroscopy, in addition to high resolution mass spectrometry. The isolated components were evaluated *in vitro* for anti-inflammatory activities for Cox-1 and Cox-2, and radical scavenging potential using $ABTS^{\cdot+}$ and DPPH test. As a result, nor seco-olean type triterpenoid **1** exhibited significant anti-inflammatory potential, while tirucallane triterpenoids **3** and **4** showed radical scavenging activities.

1. Introduction

The genus *Dysoxylum* (Meliaceae) contains about 80 species that are distributed naturally in India, Malaysia, Indonesia, Australia, and New Zealand. Among them, 11 species and one variety grow in the south of China [1,2]. The bark of many plants in this genus is known to have emetic, antiperiodic, anthelmintic and emmenagogue properties, and is used as traditional medicine for the treatment of cardiac disease [3–5]. Plants of the Meliaceae family are a rich source of structurally diversified limonoids and triterpenoids with various biological activities, which have attracted considerable interest of the natural products chemists. Previous investigation in the plants of this genus has led to the isolation of a diverse range of bioactive secondary metabolites, such as anti-tumor triterpenoid saponins [6], anti-RSV and antifeeding tetranortriterpenoids [7,8], cytotoxic diterpenoids [9] and cardiac active alkaloids. *Dysoxylum hainanense* is distributed in Guangxi Zhuang Autonomous Region, Hainan province, and Xishuangbanna, Yunnan province [10]. The present studies on chemical constituents of the EtOH extract of *D. hainanense* afforded four new triterpenoids, including two seco-olean type triterpenoids dysoxyhaine A (**1**) and dysoxyhaine B (**2**) and two tirucallane triterpenoids dysoxyhaine C (**3**) and dysoxyhaine D (**4**). The structures of these compounds were elucidated mainly by NMR spectroscopic and mass spectroscopic methods. Furthermore, all the triterpenoids were *in vitro* evaluated for their anti-inflammatory and radical scavenging effects.

2. Experimental part

2.1. General

Optical rotations were determined with a JASCO P2000 digital polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on JASCO V-650 and JASCO FT/IR-4100 spectrophotometers (JASCO Corporation, Tokyo, Japan), respectively. NMR spectra were measured on a Bruker AM-500 spectrometer (Bruker Daltonics, Billerica, Massachusetts, USA). High resolution ESI-MS spectra were obtained on a LTQ Orbitrap XL spectrometer (Thermo Fisher Scientific, Waltham MA, USA). Precoated thin-layer chromatography (TLC) plates with silica gel GF_{254} (Qingdao Haiyang Chemical Co. Ltd., Qingdao, China) were used for TLC. All solvents used were of analytical grade (Shanghai Chemical Reagents Company Ltd., Shanghai, China). Middle Chromatogram Isolated (MCI) gel CHP20P (Mitsubishi Chemical Corporation, Tokyo, Japan), Silica gel (200–300 mesh), silica gel H (Qingdao Haiyang Chemical Co. Ltd., Qingdao, China), C_{18} reversed-phase silica gel (150–200 mesh, Merck, New York, America) was used for column chromatography. Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-18 column (20 × 250 mm) and 1100 Series Multiple Wavelength detector.

2.2. Plant material

The barks of *D. hainanense* were collected from the Xishuangbangna,

* Corresponding author.
E-mail address: xdc2017@yahoo.com (D.-C. Xiang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.fitote.2017.07.012
Received 26 June 2017; Received in revised form 16 July 2017; Accepted 19 July 2017
Available online 21 July 2017

Yunnan province, China in October 2016. A specimen (201610001) was identified by one of the authors (W.T. Liu) and deposited in the Herbarium of Medical School, Jilin University, Nanjing, P R China.

2.3. Extraction and isolation

The air-dried stem bark (4.1 kg) of *D. hainanense* was ground into powder and extracted thrice with 70% EtOH. After evaporation of the EtOH, the crude extract (328 g) was partitioned between water and EtOAc. The EtOAc-soluble portion (118 g) was chromatographed on a silica gel column eluting with $CHCl_3$/MeOH (100:1, 50:1, 30:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, v/v, each 5 L) to afford fractions 1–10. Fraction 4 (7.6 g) was chromatographed on a MCI gel column and eluted with MeOH/H_2O (from 70% to 95%) to yield 6 subfractions, 4A–4F. Subfraction 4D (1.3 g) was separated by repeated column chromatography (CC) over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1, and MeOH, each 1 L), silica gel, and then preparative HPLC (MeCN/H_2O 45% to 70%), yielding **3** (31.2 mg, retention time: 15.6 min). Fraction 5 (6.2 g) was divided into eight subfractions (5A–5H) by MCI gel CC eluting with MeOH/H_2O (from 50% to 95%). Subfraction 5C (186 mg) was further separated by preparative HPLC (MeCN/H_2O, from 30% to 55%) to afford **4 (**33.5 mg, retention time: 15.3 min). Subfraction 5E (345 mg) was chromatographed on a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1, 1 L) and preparative HPLC (MeCN/H_2O, from 45% to 70%), yielding **1** (33.7 mg, retention time: 14.7 min). Separation of fraction 6 (4.2 g) by silica gel column chromatography, eluting with petroleum ether–Me_2CO (from 8:1 to 1:1), afforded five subfractions (6A–6E). Subfraction 6b (297 mg) was subjected to RP-18 (MeOH-H_2O, from 2:8 to 6:4) and Sephadex LH-20 (MeOH, 1 L) column chromatography to yield **2** (53 mg).

2.3.1. Dysoxyhaine A (1)

Colorless solid; $[\alpha]_D^{20}$ = + 241.7 (*c* = 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 285 (4.04) nm; IR (KBr) ν_{max} 3429, 2947, 1703, 1632, 1466, 1375, 1223, 835 cm^{-1}; ^1H and ^{13}C NMR see Table 1; HR-ESI-MS (pos.) *m/z* 497.3611 (calcd for $C_{31}H_{46}O_4Na$ $[M + Na]^+$, 497.3620).

2.3.2. Dysoxyhaine B (2)

White amorphous powder; $[\alpha]_D^{20}$ = + 96.1 (*c* = 0.10, MeOH); IR (KBr) ν_{max} 3431, 2960, 1701, 1466, 1383, 1263, 1176, 669 cm^{-1}; ^1H and ^{13}C NMR see Table 1; HR-ESI-MS (pos.) *m/z* 477.3569 (calcd for $C_{29}H_{49}O_5$ $[M + H]^+$, 477.3580).

2.3.3. Dysoxyhaine C (3)

White amorphous powder; $[\alpha]_D^{20}$ = + 8.7 (*c* = 0.50, MeOH); IR (KBr) ν_{max} 3501, 2951, 1711, 1635, 1460, 1373, 1243, 671 cm^{-1}; ^1H and ^{13}C NMR see Table 1; HR-ESI-MS (pos.) *m/z* 493.3283 (calcd for $C_{30}H_{46}O_4Na$ $[M + Na]^+$, 493.3294).

2.3.4. Dysoxyhaine D (4)

White amorphous powder. $[\alpha]_D^{20}$ = − 3.1 (*c* = 0.45, MeOH); IR (KBr) ν_{max} 3415, 2947, 1705, 1637, 1458, 1362, 1254, 667 cm^{-1}; ^1H and ^{13}C NMR see Table 1; HR-ESI-MS (pos.) *m/z* 495.3441 (calcd for $C_{30}H_{48}O_4Na$ $[M + Na]^+$, 495.3450).

2.4. Anti-inflammatory assay in vitro

The anti-inflammatory activities were determined according to a literature method with minor modifications [11]. The reaction system was incubated at 25 °C for 5 min, by sequential addition of the buffer, heme, test compounds, and Cox-1 or Cox-2 into the system followed by mixing with TMPD and arachidonic acid. The absorbance value was recorded at a wavelength of 590 nm after another 15 min of incubation at 25 °C. SC-560 and NS-398 were used as positive controls, which gave the inhibition of Cox-1 (63.20%) and Cox-2 (97.13%) respectively (Table 3). All cell lines were purchased from the Cell Bank of Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences (Shanghai, China).

2.5. Microplate assay for radical scavenging activity DPPH

Microplate DPPH assay was performed as described by [12]. Briefly, in a 96-well plate, successively sample dilutions (standard stocks of different samples 5 mM), in triplicate, received DPPH solution (40 μM in methanol) in a total volume of 0.2 mL and absorbance was measured at 550 nm with a microplate reader. Results were determined each 5 min until 60 min in order to evaluate kinetic behavior of the reaction. The percentage of remaining DPPH was calculated as follows: % $DPPH_{rem}$ = 100 × ($[DPPH]_{sample}/[DPPH]_{blank}$). A calibrated Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (3.9 mM initial concentration) standard curve was also made. The percentage of remaining DPPH against the standard concentration was then plotted in an exponential regression, to obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (IC_{50}).

2.6. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay

$ABTS^{\cdot+}$ radical scavenging activity was determined according to Re [12,13]. The $^{\cdot+}$ cation radical was produced by the reaction between 7 mM ABTS in H_2O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 16 h. Before usage, the $ABTS^{\cdot+}$ solution was diluted with phosphate buffer (0.05 M, pH 7.4) to get an absorbance of 0.800 ± 0.035 at 734 nm. The solution is stable for 2 days. To a 1 mL of $ABTS^{\cdot+}$ solution was added different concentrations of extracts or pure compounds solution in methanol. The mixture was incubated at 37 °C in the dark. After 30 min of incubation, the percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to a blank absorbance (methanol). All determinations were carried out at least three times, and in triplicate. The capability to scavenge the $ABTS^{\cdot+}$ radical was calculated using the following equation:

$ABTS^{\cdot+}$ scavenging effect(%) = 100 − [($A_{Sample}/A_{Control}$) × 100]

Where in $A_{Control}$ is the initial concentration of the $ABTS^{\cdot+}$ and A_{Sample} is absorbance of the remaining concentration of $ABTS^{\cdot+}$ in the presence of different compounds. Trolox was used as reference. The stock concentrations of Trolox and of different compounds tested are the same as reported in DPPH assay.

3. Results and discussion

Compound **1** was obtained as a colorless solid. The HR-ESI-MS displayed a pseudomolecular ion at *m/z* 497.3611 $[M + Na]^+$ (calcd for $C_{31}H_{46}O_4Na$, 497.3620) consistent with a molecular formula of $C_{31}H_{46}O_4$, corresponding to 9 degrees of unsaturation. The IR spectrum indicated the presence of hydroxy (3429 cm^{-1}) and carboxyl (1703 cm^{-1}) groups, as well as a C=C bond (1632 cm^{-1}). Its ^{13}C NMR exhibited 31 carbon signals including one conjugated double bond (δ_C 148.7 and 119.1; 146.8 and 121.7), one terminal double bond (δ_C 147.0 and 113.6), two carboxyl groups (δ_C 176.0 and 184.0), five sp3 quaternary carbons, two sp3 methines, nine sp3 methylenes, six methyls, and one methoxy (Table 1). The above described functionalities accounted for five out of the nine degrees of unsaturation, indicating that compound **1** possessed a tetracyclic core. The ^1H NMR displayed six singlet methyls (one at δ_H 1.78 attached on double bond) in the up-field region and two mutually coupling olefinic proton resonances at δ_H 5.83 and 5.66 (each ^1H, *J* = 6.4 Hz) in the low-field region (Table 1), which was consistent with the UV absorption band at ν_{max} = 285 nm (log ε = 4.04). The two mutually coupling olefinic proton resonances are ascribable to H-11 and H-12 respectively based on the evidence of ^1H—^1H COSY and HMBC correlations of proton signal at δ_H 5.83 with C-8, C-10, and C-13, and of proton signal at δ_H 5.66 with C-9, C-14, and C-