Fitoterapia 121 (2017) 53–57

Contents lists available at ScienceDirect

Fitoterapia

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

Cytotoxic aspidofractinine alkaloids from *Kopsia hainanensis*

Ying Wang[a], Lei Hang[b], Li Jiao[c], Hui Liu[d], Fei Li[e,*]

[a] Department of Gastroenterology, The First Hospital of Jilin University, Changchun 130021, China
[b] Department of Hepatology, The First Hospital of Jilin University, Changchun 130021, China
[c] Department of Physiology, Bethune Military Medical Academy, Shijiazhuang 050061, China
[d] Department of Field First Aid, Bethune Military Medical Academy, Shijiazhuang 050061, China
[e] Department of Endocrine Metabolism, The First Hospital of Jilin University, Changchun 130021, China

ARTICLE INFO

Keywords:
Kopsia hainanensis
Apocynaceae
Alkaloids
Cytotoxicity

ABSTRACT

Five new aspidofractinine-type alkaloids, kopsiahainins A–E (**1–5**), were isolated from a 80% EtOH extract of the leaves and stems of *Kopsia hainanensis*. Structural elucidation of all the compounds were performed by spectral methods such as 1D- and 2D-NMR, IR, UV, and HR-ESI-MS. Alkaloids **3** and **4** exhibited some cytotoxic activity against all of six tested tumor cell lines (BGC-823, HepG2, MCF-7, SGC-7901, SK-MEL-2, and SK-OV-3) with IC_{50} values of 7.3–9.5 μM and 9.2–10.6 μM, respectively.

1. Introduction

The genus *Kopsia*, belonging to the family of Apocynaceae, consists of 30 species which are distributed widely in Southeast Asia, India, China, and Australia, with the majority of the species occurring in Southeast Asia [1,2]. The roots of several *Kopsia* species are known to be used for poulticing ulcerated noses in tertiary syphilis [3]. Plants of this genus are prolific producers of alkaloids, in particular indole and bisindole alkaloids, and are well known to elaborate alkaloids with useful biological activities including antileishmanial, antimanic effects, antitumor, and antitussive activities [4–6]. Kopsia alkaloids are notable for possessing structurally intriguing molecular skeletons which were postulated to derive from known monoterpenoid indole precursors through pathways involving deep-seated rearrangements and/or loss of key fragments, such as the cage indole arbophylline [7], the three-nitrogen pentacyclic indole arboflorine [8], the tetracyclic indole mersicarpine [9], the tetracyclic quinolinic alkaloid mersilongine [10], and the pair of intriguing regioisomeric tetracyclic indoles arboricine and arboricinine [11]. One particular member of the *Kopsia* genus, *Kopsia hainanensis*, has historically been used in folk medicine for the treatment of dropsy, pharyngitis, tonsillitis and rheumatoid arthritis in China [12]. Previous studies on *K. hainanensis* resulted into the isolation a series of indole alkaloids [13,14]. In our continuous search for biologically active compounds from medicinal plants, we undertook a phytochemical investigation on the 80% ethanol extract of the aerial parts of *K. hainanensis* afforded five new aspidofractinine alkaloids, *kopsiahainins* A–E (**1–5**). This paper describes the isolation and structure elucidation of the new compounds, as well as the *in vitro* cytotoxic potential of alkaloids **1–5** against six tumor cell lines.

2. Experimental part

2.1. General

Optical rotations were determined with a JASCO P2000 digital polarimeter (JASCO Corporation, Tokyo, Japan). Ultraviolet (UV) and infrared (IR) spectra were obtained on JASCO V-650 and JASCO FT/IR-4100 spectrophotometers, respectively. The NMR spectra were recorded on a Varian Unity INOVA 500 FT-NMR spectrometer (Varian Medical Systems, Salt Lake City, UT, USA; 500 MHz for ^{1}H; 125 MHz for ^{13}C, respectively). Chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.0 ppm) and CD_3OD (δ_H 3.30 and δ_C 49.0 ppm) as internal standard. High resolution ESI-MS spectra were obtained on a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA) spectrometer. Silica gel 60 (Merck, Darmstadt, Germany, 230–400 mesh), LiChroprep RP-18 (Merck, 40–63 μm), and Sephadex LH-20 (Amersham Pharmacia Biotech., Roosendaal, The Netherlands) were used for column chromatography (CC). Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck) were used for analytical thin-layer chromatography analyses. HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector, with a Prevail (250 × 10 mm i.d.) preparative column packed with C_{18} (5 μm).

* Corresponding author.
E-mail address: lifei2727@163.com (F. Li).

http://dx.doi.org/10.1016/j.fitote.2017.06.023
Received 3 June 2017; Received in revised form 21 June 2017; Accepted 26 June 2017
Available online 27 June 2017
0367-326X/

2.2. Plant material

The leaves and stems of *K. hainanensis* were collected from the Wuzhishan County fo Hainan Province of China in June 2016. A specimen (No. 20160601) was identified by one of the authors (X. Mao) and deposited at the Natural Product Laboratory of Medical School, Jilin University, P R China.

2.3. Extraction and isolation

The air-dried and powdered aerial parts of *K. hainanensis* (8.6 kg) were cut into small pieces and were extracted with 80% EtOH (20 L × 3) at room temperature for 24 h each time. After removal of EtOH under reduced pressure at 55 °C, the aqueous brownish syrup (1 L) was suspended in H_2O (l L) and then succesively partitioned with petroleum ether (1 L × 3), chloroform (1 L × 3), and *n*-butanol (1 L × 3) to afford fractions of 41.2 g, 52.7 g, and 67.2 g, respectively. The chloroform fraction was further fractionated through a silica gel column (200–300 mesh, 10 × 80 cm, 500 g) using increasing volumes of acetone in petroleum ether (b.p. 60–90 °C) (100:1, 50:1, 30:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, v/v, each 3 L) as the eluent to give 8 fractions according to TLC analysis. Fraction 4 (petroleum ether-acetone 15:1, 3.6 g) was applied to an ODS MPLC column (100 g) and eluted with MeOH-H_2O (20:80, 30:70, 40:60, each 500 mL) to yield four subfractions (Fr. 4–1 and Fr. 4–4). Subfraction 4–2 (MeOH-H_2O, 350 mg) was purified by preparative RP-HPLC (ODS column, 250 × 20 mm) using MeOH-H_2O (25:75) as mobile phase to obtain **1** (71 mg, retention time: 13.3 min). Subfraction 4–2 (MeOH-H_2O, 350 mg) was chromatographed by a Sephadex LH-20 column eluted with MeOH-H_2O (50:50), and purifed by preparative RP-HPLC (ODS column, 250 × 20 mm) using MeOH-H_2O (30:70) as mobile phase to yield **3** (88 mg, retention time: 15.1 min)). Subfraction 4–4 (MeOH-H_2O 40:60, 99 mg) was purified by preparative RP-HPLC (ODS column, 250 × 20 mm) eluting with MeOH/H_2O (22:78) to get **4** (57 mg, retention time: 14.7 min)). Fraction 5 (petroleum ether-acetone 30:1, 1.3 g) was applied to an ODS column eluted with MeOH-H_2O (30:70, 40:60, 50:50, each 500 mL) to provide three subfractions (Fr. 5–1 and Fr. 5–3), Subfraction 5–2 (MeOH-H_2O 20:80, 226 mg) was was repeatedly chromatographed on silica gel (150 g, 60 × 2.8 cm, chloroform-methanol, 20:1 → 10:1, each 500 mL) and then purifed on a Sephadex LH-20 column eluted with MeOH-H_2O (50:50) to afford **2** (78 mg). Subfraction 5–3 was purified by preparative RP-HPLC (ODS column, 250 × 20 mm) eluting with MeOH/H_2O (20:80) to get **5** (77 mg, retention time: 15.5 min).

2.3.1. Kopsiahainin A (1)

Colorless oil; $[\alpha]_D^{20}$ − 45.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 216 (4.58), 253 (4.04), 280 (3.54), 291 (3.45) nm; IR (KBr) ν_{max}: 3349, 1743, 1659 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-FAB-MS *m/z*: 485.1563 ($C_{24}H_{25}N_2O_9$ $[M + H]^+$, calc. 485.1560).

2.3.2. Kopsiahainin B (2)

Colorless oil; $[\alpha]_D^{20}$ − 23.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε): 215 (4.58), 254 (3.98), 2811 (3.45), 291 (3.30) nm; IR (KBr) ν_{max}: 3350, 1745, 1661 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-ESI-MS *m/z*: 487.1722 ($C_{24}H_{27}N_2O_9$ $[M + H]^+$, calc. 487.1717).

2.3.3. Kopsiahainin C (3)

Colorless oil; $[\alpha]_D^{20}$ + 23.4 (*c* = 0.16, MeOH); UV (MeOH) λ_{max} (log ε): 216 (4.52), 252 (3.95), 283 (3.36), 290 (3.32) nm; IR (KBr) ν_{max}: 3391, 1743, 1667 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-FAB-MS *m/z*: 501.1513 ($C_{24}H_{25}N_2O_{10}$ $[M + H]^+$, calc. 501.1509).

2.3.4. Kopsiahainin D (4)

Colorless oil; $[\alpha]_D^{20}$ + 9.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 217 (4.48), 254 (3.92), 287 (2.82) nm; IR (KBr) ν_{max}: 3481, 3350, 1742, 1677 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-ESI-MS *m/z*: 489.1875 ($C_{24}H_{29}N_2O_9$ $[M + H]^+$, calc. 489.1873).

2.3.5. Kopsiahainin E (5)

Yellowish oil; $[\alpha]_D^{20}$ − 12.5 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε): 203 (3.59), 243 (3.01), 293 (2.62) nm; IR (KBr) ν_{max}: 3433, 3352, 1725 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; HR-ESI-MS *m/z*: 199.1923 ($C_{22}H_{27}N_2O_5$ $[M + H]^+$, calc. 199.1920).

2.4. Cytotoxicity assay in vitro

The cytotoxic activities of the isolated compounds were determined using the revised MTT method [15] against BGC-823 cells (human gastric carcinoma), HepG2 cells (Human hepatocellular carcinoma), MCF-7 cells (human breast cancer), SGC-7901 cells (human gastric adenocarcinoma), SK-MEL-2 (human skin cancer), and with SK-OV-3 (ovarian), with doxorubicin (DOX, adriamycin, Sigma Chemical Co., St. Louis, MO, USA) as positive control. Cancer cells (4×10^3 cells) suspended in 100 μL/well of DMEM medium containing 10% fetal calf serum were seeded onto a 96-well culture plate. After 24 h pre-incubation at 37 °C in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution were added and cells were incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well followed by further incubation at 37 °C for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting and was used as criteria to judge the cytotoxicity. All cell lines were purchased from Cell Bank of Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences. Other reagents were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China).

3. Results and discussion

Kopsiahainin A (**1**) was obtained as a colorless oil. The UV spectrum showed absorption maxima at 216, 253, 280, and 291 nm, indicating the presence of a dihydroindole chromophore. The IR spectrum exhibited characteristic absorption bands at 3349, 1743, and 1659 (broad) cm^{-1} which were assigned to OH, ester, and carbamate or lactam functions, respectively. The positive HR-FAB-MS spectrum displayed a pseudomolecular ion at *m/z* 485.1563 $[M + H]^+$ (calcd for $C_{24}H_{25}N_2O_9$, 485.1560) consistent with a molecular formula of $C_{24}H_{24}N_2O_9$, corresponding to 14° of unsaturation. The ^1H NMR spectrum of **1** showed two aromatic protons [δ_H 6.67 (1H, d, J = 7.4 Hz, H-9) and 6.57 (1H, d, J = 7.4 Hz, H-10)] assignable to an disubstituted indole ring, two olefinic protons at δ_H 6.18 and 6.12 (H-14 and H-15, respectively), and two methoxy signals were observed at δ_H 3.79 and 3.84, which are associated with ester and carbamate methoxy groups, respectively, from the observation of the associated carbonyl resonances at δ_C 170.4 and 154.3. The low-field singlet at δ_H 6.65 is due to the 16-OH group, while the doublet at δ_H 3.16 (J = 5.3 Hz) was assigned to the 17-OH function. The ^{13}C NMR spectrum of **1** showed the presence of a conjugated lactam carbonyl function at δ_C 162.8, in addition to the carbamate and ester carbonyl absorptions at δ_C 154.3 and 170.4, respectively. This was further confirmed from the substantial downfield shift of the olefinic H-15 resonance to δ_H 6.12, which is characteristic of the β-positioned H-atom of an α,β-unsaturated carbonyl moiety. The NMR signals indicated that **1** was an aspidofractinine-type alkaloid. The NMR data resembled that of kopsidarine [16], except for the presence of a methylenedioxy substituent (δ_H 5.91 and 5.95; δ_C 100.7). The NOESY correlations of H-6 (δ_H 2.80 and 1.50) with a pair of aromatic AB doublets at δ_H 6.67 (H-9) and 6.57 (H-10) and the HMBC correlations of proton signals of the methylenedioxy