NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2021.1984911

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Two new monoterpenoid indole alkaloids from the kernels of *Kopsia arborea*

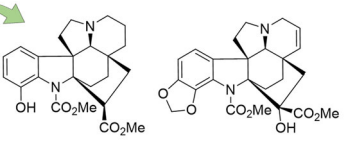
Chen Chen[a,b], Xiao Ding[b], Lingling Han[b], Wentao Zhu[b], Kepu Huang[b], Xiaojiang Hao[b] and Yu Zhang[b]

[a]School of Life Science, Yunnan University, Kunming, China; [b]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China

ABSTRACT

Two new monoterpenoid indole alkaloids, (2*R*, 7*R*, 16*R*, 20*R*, 21*S*)-12-hydroxypleiocarpine (**1**) and (2*S*, 7*R*, 16*S*, 20*R*, 21*S*)-*N*-methoxycarbonyl-11,12-methylenedioxy-$\Delta^{14,15}$-kopsinaline (**2**), along with six known alkaloids were isolated from the methanol extract of the kernels of *Kopsia arborea*. Their structures including the absolute configurations were elucidated by HRESIMS, NMR spectroscopy, and quantum computational methods. Their cytotoxicity against two human cancer cell lines were also evaluated.

Kopsia arborea



1 2

ARTICLE HISTORY
Received 11 August 2021
Accepted 17 September 2021

KEYWORDS
Apocynaceae; *Kopsia arborea*; kernels; monoterpene indole alkaloids

1. Introduction

Monoterpenoid indole alkaloids (MIAs) are characteristic components elaborated mainly by plants of Apocynaceae, Rubiaceae, and Loganiaceae families, and a series of new MIAs with structural complexity and promising biological activity have been isolated and identified in recent years (Zhang et al. 2019; Yi et al. 2020; Huo et al. 2021).

Plants of the genus *Kopsia* (Apocynaceae) are widely distributed in tropical and subtropical Asia, which are rich in monoterpene indole alkaloids and have been extensively studied (Tsiang and Li 1977; Awang et al. 1993; Kan et al. 1995; Yoganathan et al. 1995; Kam and Yoganathan 1997; Kam and Subramaniam 1998; Kam et al. 1998). *Kopsia arborea* Blume has been used to treat rheumatoid arthritis, tonsillitis, pharyngitis, and edema as a traditional medicinal plant in southern China (Zeng et al. 2017). The fruits and leaves of *K. arborea* can be used to reduce inflammation and relieve pain (Zeng et al. 2017; Xie et al. 2020). In recent years, many novel MIAs with

CONTACT Yu Zhang zhangyu@mail.kib.ac.cn

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.1984911.

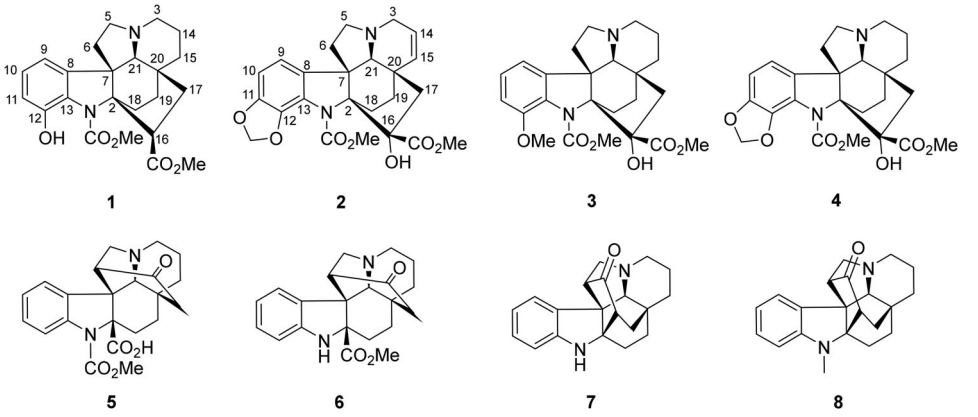


Figure 1. Molecular structures of **1**–**8** isolated from *Kopsia arborea*.

significant activity have been discovered through the study of the chemical constituents of *K. arborea* (Wang et al. 2019). The research on *K. arborea* mainly focused on the part of the twigs, leaves and fruits. In our preliminary experiment, the alkaloid constituents were mainly distributed in its kernels, and the chemical composition study of its kernels has not been reported. As a part of an ongoing search for structurally unique and bioactive MIAs (Li et al. 2018; Zhang et al. 2020; Zhu et al. 2020), two new monoterpenoid indole alkaloids, (2*R*, 7*R*, 16*R*, 20*R*, 21*S*)-12-hydroxypleiocarpine (**1**) and (2*S*, 7*R*, 16*S*, 20*R*, 21*S*)-*N*-methoxycarbonyl-11,12-methylenedioxy-$\Delta^{14,15}$-kopsinaline (**2**), along with six known alkaloids, 12-methoxy-*N*-methoxycarbonylkopsinaline (**3**), *N*-methoxycarbonyl-11,12-methylenedioxykopsinaline (**4**), chanofruticosinic acid (**5**), *N*-decarbomethoxy-chanofruticosinate (**6**), (-)-kopsanone (**7**), and *N*-methylkopsanone (**8**) were isolated from the kernels of *K. arborea*. Herein, the isolation, structural elucidation, and activity of the new isolates were reported.

2. Results and discussion

(2*R*, 7*R*, 16*R*, 20*R*, 21*S*)-12-hydroxypleiocarpine (**1**) was obtained as a white powdery substance, m.p. 259-260.5 °C, [α]24D-98.5 (*c* 0.2, MeOH), and gave a positive reaction with Dragendorff's reagent. Its molecular formula was determined as $C_{23}H_{28}N_2O_5$ by HR-ESI-MS at *m/z*: 413.2077 $[M+H]^+$ (calcd. 413.2071) corresponding to 11 degrees of unsaturation. The IR absorptions at 3445 and 1661 cm^{-1} implied the presence of hydroxyl and carbonyl functions. The ^1H and ^{13}C NMR data (Table S1) showed that compound **1** has 23 carbon signals, including two methyls, eight methylenes, five methines and eight quaternary carbons. Analysis of its NMR data manifested that **1** was highly similar to 12-methoxypleiocarpine (Kam and Sim 1998), with the only difference being that the methoxy group was replaced by a hydroxyl group. HMBC correlations of hydroxyl group (δ_H 10.40) to C-11 (δ_C 117.5), C-12 (δ_C 145.5) and C-13 (δ_C 125.7) indicated that the hydroxyl substitution was at C-12 (Figure S7). The planar structure of alkaloid **1** was further confirmed by analyzing the 2 D-NMR data (HSQC, HMBC, and ^1H-^1H COSY) as shown in Figure 1.

The relative configuration of compound **1** was deduced from the analysis of its ROESY spectra combined with the molecular modeling studies (Figure S7). The ROESY correlation of H-5a and H-17a revealed that bridges C-2-C-16-C-17-C-20 took β-configuration, whereas H-21, CH_2-18, and CH_2-19 took α-orientation. Furthermore, ROESY correlation of H-16 with H-18a and H-19a indicated that H-16 was α-oriented. Therefore, the relative configuration of compound **1** was determined as shown in Figure S7.

The absolute configuration of **1** was finally determined by time-dependent density functional theory (TDDFT) ECD calculations. The results demonstrated that the calculated ECD curve for (2*R*, 7*R*, 16*R*, 20*R*, 21*S*)-**1** matched well with its experimental ECD spectrum (Figure S11), which finally established the absolute configuration of **1** as showed in Figure 1.

(2*S*, 7*R*, 16*S*, 20*R*, 21*S*)-*N*-methoxycarbonyl-11,12-methylenedioxy-$\Delta^{14,15}$-kopsinaline (**2**) was obtained as a white powdery substance with m.p. 268-272 °C and [α]24D = 18.7 (*c* 0.1, MeOH), it's molecular formula was determined as $C_{24}H_{26}N_2O_7$ by HR-ESI-MS at *m/z*: 455.1823 $[M+H]^+$ (calcd. 455.1813) corresponding to 13 degrees of unsaturation. IR absorption bands at 3440 and 1681 cm^{-1} suggested the presence of hydroxyl and carbonyl functions. The ^1H and ^{13}C NMR data (Table S1) comprised 24 carbon signals, including two methyls, seven methylenes, five methines and ten quaternary carbons. Detailed analysis of its NMR data indicated that **2** might be dehydrogenated derivative of **4** (Kam et al. 1999). The ^1H-^1H COSY correlations of H-3a (δ_H 3.40)-H-14 (δ_H 5.69)-H-15 (δ_H 5.52) and HMBC correlations of H-3a to C-15 (δ_C 132.6), and of H-14 to C-20 (δ_C 36.5) confirmed the double bond was located at C-14,15 (Figure S18). The other partial structures were identical with **4**, which was confirmed by further 2 D NMR (HSQC, HMBC, and ^1H-^1H COSY) experiments.

The relative configuration of compound **2** was identical with those of **1**, as established by analyzing their similar ROESY spectrum (Figure S18). However, the relative configuration of 16-OH was unassigned since the absence of ROESY correlation of 16-OH with any protons. Hence, alkaloids **2** has two possible isomers, namely, (2*R**, 7*R**,16*S**,20*R**, 21*S**)-**2** or (2*R**, 7*R**, 16*R**, 20*R**, 21*S**)-**2**. To confirm the above elucidation, the ^{13}C NMR data were computed by using DFT studies at the B3LYP/6–311 + G(2d, p) level for the two isomers (Forsyth and Sebag 1997). The observed experimental ^{13}C NMR data matched up to the calculated data for the isomer (2*R**, 7*R**, 16*S**, 20*R**, 21*S**)-**2**, thus indicating a 16*S** configuration (Table S2). Furthermore, by comparing the similar ECD spectrum of **1** with **2**, the absolute configuration of **2** could be unambiguously defined as 2*R*, 7*R*, 16*S*, 20*R*, 21*S*.

Six known alkaloids were identified as 12-methoxy-*N*-methoxycarbonylkopsinaline (**3**) (Kam and Sim 1998), *N*-methoxycarbonyl-11,12-methylenedioxykopsinaline (**4**) (Kam et al. 1999), chanofruticosinic acid (**5**) (Zeng et al. 2017), *N*-decarbomethoxy-chanofruticosinate (**6**) (Chen et al. 1981), (-)-kopsanone (**7**) (Jones et al. 2011), *N*-methylkopsanone (**8**) (Kuehne and Seaton 1985) by comparing their spectral data with those reported in the literatures.

The two new alkaloids were evaluated for their cytotoxicities against two human cancer cell lines HepG-2 (hepatocellular carcinoma) and CNE-2 (nasopharyngeal carcinoma) using MTT method (Stockert et al. 2012). However, both of them were inactive (IC_{50}>40 μM).

3. Experimental

3.1. General experimental procedures

The UV data were measured using a Shimadzu UV-2410A spectrophotometer. The 1 D and 2 D NMR spectra were performed on Bruker 500 MHz spectrometers with TMS as the internal standard. Optical rotations were recorded on a JASCO P-1020 digital polarimeter, and the ECD spectral data were performed on the optical physical chirascan spectrometer. The values of melting points were determined by the UTM-300 micro-melting point apparatus. IR spectra data were done with a Bio-Rad FTS-135 spectrometer from KBr pellets. Column chromatography was performed on silica gel H (10-40 μm, Qingdao Marine Chemical Inc., China) and silica gel (60-80, 200-300 and 300-400 mesh, Qingdao Marine Chemical Inc., China), MCI gel 20 P (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (40-70 μm, Amersham Pharmacia Biotech AB). Semi-preparative HPLC was performed on a Waters X-bridge (5 μm; 10 mm $\times$ 150 mm), C_{18} reversed-phase column. The visualizing reagent was potassium bismuth iodide.

3.2. Plant material

The dried kernels of *K. arborea* were collected in campus of Sun Yat-sen University, Guangdong Province, China in December 2017 and were identified by Dr. Gui-Hua Tang. The voucher specimen (ZY20171212) is preserved in the Key Laboratory of Western Phytochemistry and Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences (CAS).

3.3. Extraction and isolation

The dried powdered kernels of *K. arborea* (1.2 kg) were extracted 3 times (4, 3 and 3 h each time) with methanol and then distill under reduced pressure to recover the methanol to obtain the extract. The extract was diluted with water and the pH was adjusted to 2-3 with 10% hydrochloric acid solution, and then extracted 3 times with petroleum ether. The aqueous phase was basified to 10-11 with 10% NaOH, and then extracted with dichloromethane to obtain 154 g of crude alkaloid. The crude alkaloid (154 g) was separated on silica gel column chromatography (300-400 mesh), and eluted with a petroleum ether/acetone (1:0→0:1) gradient, and then dichloromethane/methanol (40:1→1: 1) gradient elution, to obtain 5 main fractions (Fr. A-Fr. E). Fr. E (46.7 g) was separated on a reversed-phase C18 column and eluted with MeOH/H_2O (20:80 → 100:0, v/v) to obtain the subfraction (Fr. EI- Fr. EIV). The subfraction EIII (7.8 g) was further separated on a RP-C18 column and eluted with MeOH/H_2O (30:70 → 100:0, v/v), followed by semi-preparative HPLC using a YMC-C_{18} column with CH_3CN/H_2O (68:32) to give compounds **5** (2.9 mg, t_R= 34.5 min), **6** (2.0 mg, t_R= 36.0 min), **7** (4.3 mg, t_R= 28.5 min) and **8** (10.8 mg, t_R= 15.0 min). The subfraction EIV (2.9 g) was further separated by Sephadex LH-20(methanol), and then subjected to silica gel column chromatography, using petroleum ether/acetone (20: 1-1:1, v/v) elution, followed by semi-preparative HPLC using a Waters X-Bridge C_{18} (10 $\times$ 250 mm, 5 μm)

column with CH_3CN/H_2O (42:58, 0.1% v/v diethylamine) to give compounds **1** (4.2 mg, t_R= 15.0 min), **2** (11.3 mg, t_R= 18.0 min), **3** (35.3 mg, t_R= 22.0 min) and **4** (40.0 mg, t_R= 26.5 min).

3.4. Activity

The two new alkaloids were evaluated for their cytotoxicities against two human cancer cell lines HepG-2 (hepatocellular carcinoma) and CNE-2 (nasopharyngeal carcinoma) using MTT method, with an IC_{50}>40 μM, respectively.

4. Conclusions

In this study, two new monoterpenoid indole alkaloids, (2*R*, 7*R*, 16*R*, 20*R*, 21*S*)-12-hydroxypleiocarpine (**1**) and (2*S*, 7*R*, 16*S*, 20*R*, 21*S*)-*N*-methoxycarbonyl-11,12-methylenedioxy-$\Delta^{14,15}$-kopsinaline (**2**), along with six known alkaloids, 12-methoxy-*N*-methoxycarbonylkopsinaline (**3**), *N*-methoxycarbonyl-11,12-methylenedioxykopsinaline (**4**), chanofruticosinic acid (**5**), *N*-decarbomethoxy-chanofruticosinate (**6**), (-)-kopsanone (**7**), *N*-methylkopsanone (**8**), were isolated from the methanol extract of the kernels of *K. arborea*. Their structures including absolute configurations were elucidated by a combination of MS, NMR, and quantum computational methods (ECD and ^{13}C NMR calculation). Their cytotoxicity was also evaluated, and both of them were inactive. The diversity of chemical components provides a basis for understanding the phytochemical taxonomy.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

We are grateful for the National Science Foundation of China (81874295), and Top Young Talent of the Ten Thousand Talents Program of Yunnan (to Y. Zhang) for financial support.

References

Awang K, Thoison O, Hadi AHA, Pais M, Sevenet T. 1993. Alkaloids from *Kopsia singapurensis*. Nat Prod Lett. 3(4):283–289.

Chen WS, Li SH, Kirfel A, Will G, Breitmaier E. 1981. Three indoline alkaloids from *Kopsia officinalis* Tsiang. Liebigs Ann Chem. 1981(10):1886–1892.

Forsyth DA, Sebag AB. 1997. Computed C-13 NMR chemical shifts via empirically scaled GIAO shieldings and molecular mechanics geometries. Conformation and configuration from C-13 shifts. J Am Chem Soc. 119(40):9483–9494.

Huo ZQ, Zhao Q, Zhu WT, Hao XJ, Zhang Y. 2021. Bousmekines A-E, new alkaloids from two *Bousigonia* species: B.angustifolia and B. mekongensis. Nat Prod Bioprospect. 11(2):207–213.

Jones SB, Simmons B, Mastracchio A, Macmillan DWC. 2011. Collective synthesis of natural products by means of organocascade catalysis. Nature. 475(7355):183–188.

Kam TS, Sim KM. 1998. Alkaloid from *Kopsia griffithii*. Phytochemistry. 47(1):145–147.

Kam TS, Subramaniam G, Wei C. 1998. 19(*R*)-hydroxyeburnamine and 19(*R*)-hydroxy-isoeburnamine from *Kopsia dasyrachis*. Nat Prod Lett. 12(4):293–298.

Kam TS, Subramaniam G. 1998. Oxo-derivatives of kopsine- and rhazinilam-type alkaloids from anrith Borneo *Kopsia*. Nat Prod Lett. 11(2):131–136.

Kam TS, Yoganathan K. 1997. Nitaphylline, a new bisindole alkaloid from *Kopsia*. Nat Prod Lett. 10(1):69–74.

Kam YS, Subramaniam G, Chen W. 1999. Alkaloids from *Kopsia dasyrachis*. Phytocehmistry. 51(1): 159–169.

Kan C, Deverre JR, Sevenet T, Quirion JC, Husson HP. 1995. Indole alkaloids from *Kopsia deverrei*. Nat Prod Lett. 7(4):275–281.

Kuehne ME, Seaton PJ. 1985. Studies in biomimetic alkaloid syntheses. 13. Total syntheses of racemic, pleiocarpine, pleiocarpinine, kopsinine, *N*-methylkposanone, and kopsanone. J Org Chem. 50(24):4790–4796.

Li Y, Zhang Y, Gan QW, Xu M, Ding X, Tang GH, Liang JJ, Liu K, Liu XZ, Wang X, et al. 2018. *C. elegans*-based screen identifies lysosome-damaging alkaloids that induce STAT3-dependent lysosomal cell death. Protein Cell. 9(12):1013–1026.

Stockert JC, Blazquez-Castro A, Canete M, Horobin RW, Villanueva A. 2012. MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. Acta Histochem. 114(8):785–796.

Tsiang Y, Li PY. 1977. Flora of China. Beijing: Science Press. Vol. 63; p. 41–44.

Wang ZW, Guo CR, Lin YL, Yan HJ, Mu Y, Geng YL, Liu W, Wang X. 2019. Chemical constituents of *Kopsia officinalis* and their antagonizing high glucose-evoked podocyte injury activity. Fitoterapia. 137:104258.

Xie TZ, Zhao YL, He JJ, Zhao LX, Wei X, Liu YP, Luo XD. 2020. Monoterpenoid indole alkaloids from the stems of Kopsia officinalis. Fitoterapia. 143:104547.

Yi W-F, Ding X, Chen Y-Z, Adelakun TA, Zhang Y, Hao X-J. 2020. Tabernaesines A-I, cytotoxic aspidosperma-aspidosperma-type bisindole alkaloids from *Tabernaemontana pachysiphon*. J Nat Prod. 83(11):3215–3222.

Yoganathan K, Wong WH, Kam TS. 1995. Novel cage indole alkaloidal artefacts containing an α-aminoketone moiety from a *Kopsia* species. Nat Prod Lett. 5(4):309–314.

Zeng T, Wu XY, Yang SX, Lai WC, Shi SD, Zou Q, Liu Y, Li LM. 2017. Monoterpenoid indole alkaloids from *Kopsia officinalis* and the immunosuppressive activity of Rhazinilam. J Nat Prod. 80(4):864–871.

Zhang Y, Ding X, Shao S, Guo LL, Zhao Q, Hao XJ, He HP. 2019. Melocochines A and B, two alkaloids from the fruits of *Melodinus cochinchinensis*. Org Lett. 21(22):9272–9275.

Zhang Y, Ding X, Yuan YX, Guo LL, Hao XJ. 2020. Cytotoxic monoterpenoids indole alkaloids from *Tabernaemontana corymbosa* as potent autophagy inhibitors by the attenuation of lysosomal acidification. J Nat Prod. 83(5):1432–1439.

Zhu WT, Zhao Q, Huo ZQ, Hao XJ, Yang M, Zhang Y. 2020. Taberdivamines A and B, two new quaternary indole alkaloids from *Tabernaemontana divaricata*. Tetrahedron Lett. 61(44): 152400.