Camganoids A and B, two new sesquiterpenes with different carbon skeletons isolated from fruits of Cinnamomum migao

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A B S T R A C T

Objective: To isolate and identify the undescribed compounds from the fruits of Cinnamomum migao and evaluate its nitric oxide inhibition potential.

Methods: The chromatographic techniques of silica gel, Sephadex, and HPLC were used for isolation and purification of the compounds, while HR-ESI-MS, 1D NMR, 2D NMR, ECD, and X-ray diffraction techniques were used to characterize and confirm the isolated compounds. Moreover, the anti-inflammatory activity of the isolated compounds was carried out to check inhibitory potential against the production of nitric oxide with RAW264.7 cells stimulated by LPS.

Results: Camganoid A (1), a novel sesquiterpene possessing an unprecedented skeleton, and camganoid B (2), containing a unique eight-membered sesquiterpene moiety with a new carbon skeleton, were isolated and identified from the fruits of C. migao. The absolute configurations of 1 and 2 were confirmed by single crystal X-ray diffraction and electronic circular dichroism (ECD) calculations. Among these compounds, compound 1 exhibited potent inhibitory activity against the production of nitric oxide with IC50 value of 4.59 μmol/L in RAW264.7 cells stimulated by LPS.

Conclusion: The isolation of two new skeletons from the fruits part of C. migao possessed unique skeletons which have not been reported before.

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1. Introduction

The genus Cinnamomum belongs to the family Lauraceae and is signified by evergreen trees and shrubs (Asadollahi et al., 2019). Approximately 250 species have been reported worldwide. These species are mostly dispersed in tropical and subtropical regions of Australia, Southeast Asia, North, South, and Central America. In China, about 46 species have been reported so far, and are mainly distributed in southern regions (Muhammad et al., 2020). In China, the most abundant species have been reported in Yunnan, Guangdong, Guizhou, and Sichuan provinces (Kumar et al., 2019; Zeng et al., 2014). The genus Cinnamomum has been reported to contain alkaloids, terpenoids, flavonoids, and volatile oils (Guoruo et al., 2017, 2018; Lin et al., 2008; Muhammad et al., 2021; Zhou et al., 2019, 2017). Pharmacologically Cinnamomum has abundant biological activities including analgesic, anti-tumor, anti-inflammatory, anti-HIV, anti-arrhythmic, and antibacterial activities (De Oliveira et al., 2015; Liu et al., 2018; Wu et al., 2020). Cinnamomum migao H. W. Li is an endemic medicinal woody plant species primarily distributed in Southwestern China. It has been reported with significant phytochemicals, such as terpenoids, phenolics, flavonoids, and has contributed to autotoxicity and allelopathic effects (Huang et al., 2019; Li & Wang, 2003). The fruits of C. migao are very effective for gastrointestinal and cerebrovascular diseases. It has long been used as traditional Miao medicine in China (Lin et al., 2008). This work is the continuation of our previous work which reported the isolation of some guaiane type sesquiterpenoids from the same species (Muhammad et al., 2020; Muhammad et al., 2021). All previous researches were only focused on the twigs and leaves of this plant. These previous results encouraged us to search for other novel natural compounds with promising bioactivities from the fruits of C. migao, which resulted in two new sesquiterpenes. Thus, camganoids A and B (1 and 2, Fig. 1), were isolated and identified from the fruits of C. migao. Compounds 1 and 2 exhibited two unique carbon skeletons.

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In this paper, we are reporting the complete details of the isolation, structural elucidation and biological activities of 1 and 2.

2. Materials and methods

2.1. General experimental procedures

HSGF254 silica gel plates (10–40 μm, Yantai, China) were used for TLC analysis. Silica gel (100–200 mesh, Yantai, China), silica gel H (10–40 μm, Qingdao, China), and Sephadex LH-20 (Pharmacia Co., Ltd.) were utilized as column chromatography materials. The Shimadzu LC-6AD series equipped with an SPD-20 spectrophotometer (Shimadzu, Co., ltd.) were utilized as column chromatography materials. The structural elucidation and biological activities of Camganoid A (1); colorless prismatic crystal; for 1H NMR and 13C NMR spectroscopic data, see Table 1; HR-ESI-MS (positive): m/z 253.1801 [M + H]+ (calcld for 253.1804).

Camganoid B (2); colorless oil; for 1H NMR and 13C NMR spectroscopic data, see Table 1; HR-ESI-MS (positive): m/z 291.1558 [M + Na]+ (calcld for 291.1572).

2.2. Plant materials

The fruits of C. migao were collected in Guizhou Province, China, in October 2018. The identification of the plant material was authenticated by Prof. Huizi Jin, School of Pharmacy, Shanghai Jiao Tong University. A reference sample (No. 201810DGMJZ) has been deposited in the School of Pharmacy, Shanghai Jiao Tong University.

2.3. Extraction and isolation

The dried fruits of C. migao (30.0 kg) were powdered and extracted with 95% ethanol three times at room temperature and evaporated under normal pressure to get crude extraction (3764.1 g). The crude extract was further partitioned with petroleum ether and ethyl acetate to give three fractions. The EtOAc fraction (500 g) was subjected to a silica gel column chromatography and eluted with a step gradient of PE-EA (100:0-0:100, volume percentage) to yield ten fractions (PE-1–PE-10). Then PE-2 (2.3 g) was further subjected to silica gel column chromatography and eluted with a step gradient of PE-EA (50:1–10:1, volume percentage), which finally got three sub-fractions (PE-2-1–PE-2-3). The sub-fraction PE-2-2 was subjected to preparative HPLC (MeOH-H2O, 60:40) to attain compound 2 (40.8 mg). PE-2-3 (0.5 g) was further isolated by silica gel column chromatography (200–400 mesh) with PE-EA (10:1–5:1, volume percentage) and got two sub-fractions (PE-2-3-1–PE-2-3-2). The subfraction PE-2-3-1 (0.1 g) was purified by PTLC (PE-EA = 10:1) to obtain compound 1 (10.5 mg).

2.4. Spectroscopic data of new compounds

Table 1

| No. | δC (ppm) | J (Hz) | δC (ppm) |
|-----|----------|--------|----------|
| 1   | 43.4     | 2.03 (7.6) | 85.1     |
| 2   | 31.3     | 2.46 (13.3) | 46.5     |
| 3   | 113.7    |        | 214.4    |
| 4   | 92.9     |        | 46.0     |
| 5   | 29.8     | 2.39 m | 29.7     |
| 6   | 28.7     | 1.46 dd (15.3, 7.2) | 33.5    |
| 7   | 25.1     | 1.90 m | 171.7    |
| 8   | 81.8     | 1.90 m | 171.7    |
| 9   | 21.9     | 1.72 m | 21.9 m   |
| 10  | 36.1     | 1.82 m | 42.0     |
| 11  | 109.4    |        | 208.0    |
| 12  | 19.3     | 1.63 s | 20.8     |
| 13  | 16.5     | 0.99 d (7.0) | 26.1     |
| 14  | 24.8     | 1.28 s | 30.1     |
| 15  | 30.3     | 1.32 s | 17.7     |

* δ in 1 × 10−6; J in Hz within parentheses; Measured at 125 MHz for 13C NMR and 500 MHz for 1H NMR in CDC6.

2.5. Computational details

The theoretical calculation of compound 1 was performed using Gaussian 09. The geometries generated based on NMR were conducted by Mest ReNova 10.0.

2.6. X-ray crystallographic analysis

Colorless prismatic crystals of 1 were obtained from acetone. Crystal data were obtained on a Bruker D8 VENTURE with a graphite monochromator with Cu Kα radiation (λ = 1.54178 Å) at 296 K. The structure was solved by direct methods using the SHELXS-97 and expanded using difference Fourier techniques, refined with the SHELXL-97. Orthorhombic crystal of C15H24O3, M = 252.34, space group P21212; cell dimensions a = 14.4039(6) Å, alpha = 90 deg.; b = 16.5970(7) Å, beta = 90 deg. c = 5.7974(3) Å, gamma = 90 deg.; volume 1385.94(11) Å3; Z = 4, calculated density 1.209 mg m−3; crystal size 0.180 × 0.200 × 0.250 mm; absorption coefficient 0.657 mm−1 F(000) = 552, beta range for data collection 4.064 to 69.202 deg.: Final R indices [I > 2σ(I)]; R1 = 0.0400, wR2 = 0.1074; R indices (all data) R1 = 0.0478, wR2 = 0.1074; Absolute structure parameter –0.03(12). Crystallographic data for 1 have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1971934). Copies of the data can be obtained free of charge via https://www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, U.K. [fax (+44) 1223–336-033; or e-mail: deposit@ccdc.cam.ac.uk].

Fig. 1. Chemical structures of compounds 1 and 2.
2.7. Bioassay for nitric oxide production

Cells were plated onto 96-well plates (4 × 10^4 cell/well) and pretreated with 100 µL of different concentrations of compounds with twofold concentration dilution for 1 h before stimulation with 100 ng/mL (for RAW 264.7) of LPS. Compounds started with a maximum dilution of 100 µmol/L. After incubation for 24 h, an equal volume (50 µL) of the supernatant was mixed with Griess Reagent (Beyotime Institute of Biotechnology, China) in a 96-well plate. The mixture was allowed to react for 15 min, and the release of nitric oxide was measured at 540 nm on a Flexstation 3 (Muhammad et al., 2021).

2.8. MTT assay for cell viability

Cells were seeded in 96-well plates at (1 × 10^4) cell/well. After 24 h, the cells were treated with 100 µL of different concentrations of compounds (10 µmol/L, 50 µmol/L) for 24 h. Subsequently, 10 µL of 5 mg/mL MTT in DD water was added to each well, and the cells were incubated for 4 h. The medium was removed and resolved with 100 µL/well DMSO. The optical density was measured at 490 nm on a Flexstation 3 (Molecular Devices, Silicon Valley, CA, USA).

2.9. Statistical analysis

The data were obtained from three independent experiments and expressed as the means ± SEM. All statistical analyses were calculated by the GraphPad Prime 7 Software (GraphPad, Avenida, CA, USA).

3. Results and discussion

3.1. Structure identification of new compounds

Compound 1 was obtained as a colorless prismatic crystal. The molecular formula C_{15}H_{24}O_{4} was established by HR-ESI-MS: m/z 253.1801 [M + H]+ (calcd for C_{15}H_{24}O_{3}H+, 253.1804), requiring four degrees of unsaturation. The absolute configuration of 1 was confirmed by single-crystal X-ray diffraction (Fig. 3).

The 1H NMR spectrum (Table 1) of 1 exhibited 24 protons, all of which were in the high-field region (δ_H 0.99–2.46 × 10^{-5}). The 1H NMR showed four methyl groups including one doublet at δ_H 0.99 (3H, d, J = 7, H-13), three singlets at δ_H 1.28 (3H, s, H-14), 1.32 (3H, s, H-15), 1.63 (3H, s, H-12), five methylene groups at [δ_H 2.46 (1H, d, J = 13.3, H-2a), 1.96 (1H, d, J = 13.3, H-2b)], [δ_H 1.90 (1H, m, H-7a), 1.60 (1H, m, H-7b)], [δ_H 1.82 (1H, m, H-10a)], 1.60 (1H, m, H-10b)], [δ_H 1.72 (1H, m, H-9a), 1.64 (1H, m, H-9b)]. [δ_H 1.46 (1H, dd, J = 15.3, 7.2, H-6a), 1.24 (1H, m, H-6b)] and two methine’s at δ_H 2.03 (1H, t, J = 7.6, H-1) and δ_H 2.39 (1H, m, H-5).

Analysis of 13C NMR and DEPT spectra showed that 1 comprises 15 carbons corresponding to four methyl’s at [δ_C 30.1, 26.1, 20.8, 17.7], five methylenes at [δ_C 42.0, 39.5, 33.5, 29.7, 24.0], two methine’s at [δ_C 46.5, 46.0], one oxygen-bonded carbon and three carbonyl carbons at [δ_C 214.4, 208.0, 171.7, 85.1]. The 1H–1H COSY correlations at H-2/H-3 and the HMBC cross-correlations from H-2 to C-1, H-3, C-4 indicated that H-2, H-3 are on the same face of the ring. The downfield chemical shifts of C-3 and C-11 indicated the presence of oxygen molecule which are directly bonded to their adjacent methylene to form the scaffold.

Fig. 2. Selected NMR correlations of compound 1.

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3.2. Structure characterization of key compounds

Compound 2 was obtained as a colorless oil. The molecular formula C_{15}H_{24}O_{4} of compound 2 was established from HR-ESI-MS [M + Na]+ at m/z 291.1558; calcld 291.1572), requiring four degrees of unsaturation. Likewise, compound 1, the 1H NMR spectrum (Table 1) of compound 2 also showed 24 protons in the high-field region (δ_H 1.09–2.60 × 10^{-6}). These signals were corresponding to four methyl groups including one doublet at δ_H 1.09 (3H, d, J = 7, H-13), three singlets at δ_H 1.26 (3H, s, H-12), 1.61 (3H, s, H-13), 2.15 (3H, s, H-14), five methylenes at [δ_H 2.53 (2H, m, H-10)], [δ_H 2.51 (1H, m, H-8a), 1.97 (1H, m, H-8b)], [δ_H 2.36 (1H, m, H-6a), 2.19 (1H, m, H-6b)], [δ_H 2.04 (1H, m, H-5a), 1.75 (1H, m, H-5b)], [δ_H 1.67 (1H, m, H-9a), 1.29 (H, m, H-9b)] and two methine’s at δ_H 2.51 (1H, m, H-2) and 2.60 (1H, m, H-4). The 13C NMR and DEPT spectra exhibited 15 carbons corresponding to four isolated methyl’s at [δ_C 30.1, 26.1, 20.8, 17.7], five methylenes at [δ_C 42.0, 39.5, 33.5, 29.7, 24.0], two methine’s at [δ_C 46.5, 46.0], one oxygen-bonded carbon and three carbonyl carbons at [δ_C 214.4, 208.0, 171.7, 85.1]. The 1H–1H COSY correlations at H-2/H-3/H-4/H-10, and the HMBC cross-correlations from H-3 to C-10/C-11, signified that 2 contains a side chain [CH_{3}–CO–CH_{2}–CH_{2}–CH_{2}–] bonded to C-2 (Fig. 4). In addition, the 1H–1H COSY correlations from H-6/H-7/H-9/H-10, and the HMBC cross-correlations from H-9 to C-3/C-4/C-5, H-2 cross signals to C-3/C-4/C-5, H-12 to C-4/C-5, H-13 to C-4/C-5, H-5 to C-7 specified that 2 contains a group [CH_{3}–C–CH_{2}–CH_{2}–] group at C-4. The key NOESY correlations from H-12 to H-13 indicated that H-12, H-13 are on the same face of the ring.

Fig. 3. X-ray structure of compound 1.
[(CH₃)₂–C–CH–CO–CH(CH₃)–CH₂–CH₂–CO–]. These correlated signals also specified the position of different functional groups along the ring. The chemical shift of C-1 (δC 85.1), confirmed the attachment of C-1 to an oxygen molecule and in turn, this oxygen was connected to carbonyl carbon at C-7 (δC 171.7). As this compound contains four degrees of hydrogen deficiency, it must have a ring. There should be an oxygen atom between C-1 and C-7 to form an eight-membered ring. Accordingly, the planar structure of 2 was determined as shown in Fig. 4. The relative stereochemistry of 2 was determined by NOESY experiments. The key NOESY correlations at H-2 to H-15 implied they were in the same face of the ring and assigned as β-oriented. To determine the absolute configuration, the CD spectrum of 2 was measured, and the spectrum was dominated by a strong negative Cotton effect at λₘₐₓ = 225 nm (Fig. 5) for the n → π⁺ transition of the lactone eight ring moiety as well as a strong positive Cotton effect at λₘₐₓ = 285 nm for the n → π⁺ transition of the ester carbonyl group. Finally, the calculation of a theoretical electronic circular dichroism (ECD) in acetonitrile, determines the absolute configuration of this natural compound. In CH₃CN, the theoretical ECD spectra of 2 and its enantiomer were calculated and compared to the experimental ECD spectrum of 2 (Fig. 5). Thus, an exactly similar pattern of the experimental and calculated ECD curve revealed the absolute configuration of 2 as 2R, 4S and was trivially named as camganoid B.

3.2. Anti-inflammatory activity

Since nitric oxide plays a significant role in causing inflammation, camganoids A and B, isolated and identified from C. migao were therefore tested for the inhibitory activity against the production of NO in RAW264.7 cells stimulated by lipopolysaccharide (LPS) (Choi & Hwang, 2004). Among these compounds, 1 exhibited a relatively high inhibitory effect against the production of NO, with an IC₅₀ value of 4.59 μmol/L (Fig. 6) while compound 2 showed no activity even at the concentration of 20 μmol/L. No significant cytotoxicity was detected by the tested compounds as determined by MTT assay.

4. Conclusion

In summary, camganoids A and B were isolated from the fruits of C. migao. For the first time, these two new skeletons have been isolated from the fruits part of C. migao. Both were confirmed as new compounds and possessed two unique skeletons that had not been reported before. The absolute configuration of 1 was established by single-crystal X-ray diffraction, while the absolute configuration of 2 was determined by electronic circular dichroism (ECD) calculations. Additionally, these compounds were tested for the inhibitory activity against the production of NO in RAW264.7 cells stimulated by lipopolysaccharide (LPS). Compound 1 exhibited potent activity against the production of nitric oxide. The potent anti-inflammatory potential of compound 1 against NO is possibly due to the close arrangements of methyl’s group with adjacent oxygen and also the chiral centers are present at a different position compared to compound 2.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chmed.2021.09.016.
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