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Dibenzocyclooctadiene lignans from Kadsura heteroclita

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Kadsuphilol C (PubChem CID: 102471193)
Kadsuphilol R (PubChem CID: 102484884)
Heteroclitin P (PubChem CID: 101845163)
Kadsuphilol T (PubChem CID: 102484886)
Heteroclitin Q (PubChem CID: 101845164)
Kadsuphilin F (PubChem CID: 102155783)
(+)-Gomisin K_3 (PubChem CID: 73057)

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ABSTRACT

Sixteen dibenzocyclooctadiene lignans, including eight new ones, kadheterins A–H (**1–8**), and a new natural product, 9-benzoyloxy-gomisin B (**9**), were isolated from the stems of *K. heteroclita*. The structures of **1–9** were elucidated by extensive spectroscopic methods, and their absolute configurations were determined by combining CD with ROESY experiments. Additionally, the absolute configuration of **1** was further confirmed by single crystal X-ray crystallographic analysis. *In vitro* activity assays showed that **1** exhibited moderate cytotoxicity against HL-60 with IC_{50} value at 14.59 μM.

1. Introduction

Kadsura heteroclita (Roxb.) Craib is a climbing woody liana belonging to the family Schisandraceae and is mainly distributed in southern mainland China [1,2]. The stems of *Kadsura heteroclita* are known as one of the major source of "Dian-ji-xue-teng", which is a traditional Chinese medicine for the treatment of menstrual irregularities, dysmenorrhea, numb hands and feet, rheumatoid arthritis, and blood deficiency [3]. Previous phytochemical studies on this species have led to the isolation of several types of secondary metabolites, such as lignans [1,3–6], lanostane and cycloartane triterpenoids [7–10], and sesquiterpenoids [11]. Lignans, especially dibenzocyclooctadiene lignans from *K. heteroclita*, have been found to possess various beneficial pharmacological effects such as anti-HIV [1], anti-lipid peroxidative [5,12], and PAF receptor antagonistic activities [4,13]. Furthermore, some triterpenoids possess antitumor and cholesterol-biosynthesis inhibition activities [8,9,14].

In order to discover novel bioactive natural compounds, *K. heteroclita* indigenous to Xishuangbanna Autonomous Prefecture, Yunnan Province, People's Republic of China, was phytochemically investigated. This paper reports on the isolation and structure elucidation of eight new dibenzocyclooctadiene lignans, kadheterins A–H (**1–8**), and a new natural product, 9-benzoyloxy-gomisin B (**9**) (Fig. 1). The structures of **1–9** were established by extensive spectroscopic methods, including IR, UV, 1D and 2D NMR experiments (^1H–^1H COSY, HSQC, HMBC, ROESY), and their absolute configurations were determined by CD combined with ROESY experiments. In addition, the structure and stereochemistry of **1** were further confirmed by single crystal X-ray diffraction analysis. Compounds **1–4**, **6**, and **7** were tested for their cytotoxicities against five tumor cell lines by MTS assay, and **1** was also tested for inhibitory activity against NO production in LPS-stimulated RAW264.7 cells. The results indicated that **1** exhibited moderate cytotoxic activity against HL-60.

2. Experimental

2.1. General experimental procedures

X-ray data were collected using a Bruker APEX DUO instrument. Melting points were obtained on an XRC-1 apparatus and were uncorrected. NMR spectra were recorded on a Bruker DRX 500, 600, or 800 spectrometer using TMS (or $CDCl_3$) as internal standard. Chemical shifts (δ) are expressed in ppm relative to the TMS (or $CDCl_3$) signals. HRESIMS was performed on an API QSTAR Pulsar i

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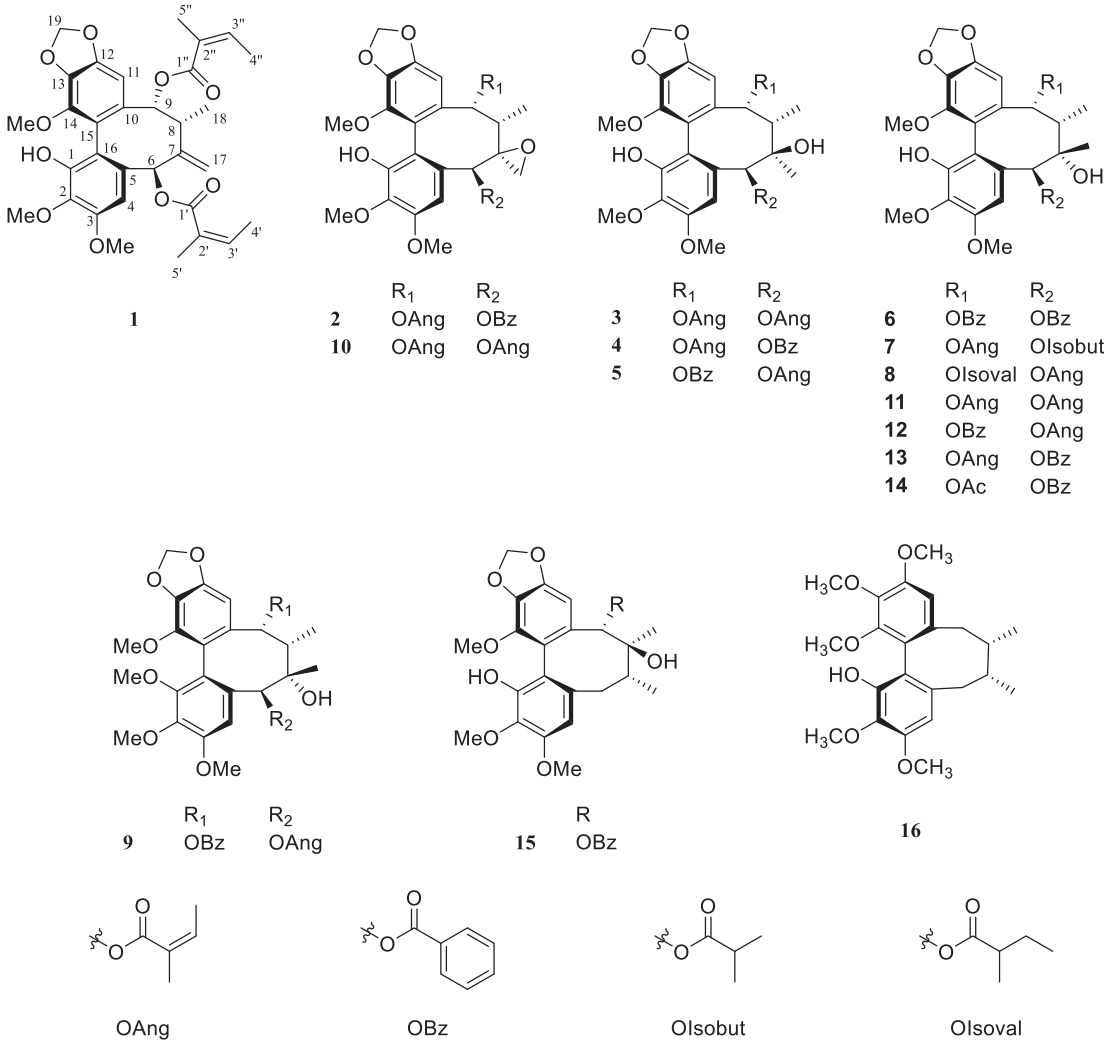

Fig. 1. The structures of compounds **1–16**.

spectrometer. UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer, IR spectra on a Bruker Tensor-27 FT-IR spectrometer using KBr pellets, and optical rotations in MeOH with Horiba SEPA-300 and JASCO P-1020 polarimeters. Experimental CD spectra were recorded on a Chirascan instrument. Column chromatography (CC) was performed with silica gel (100–200 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Preparative HPLC was performed on an Agilent 1260 preparative liquid chromatograph with a Zorbax SB-C18 (21.2 mm × 250 mm) column. Semi-preparative HPLC was performed on an Agilent 1200 liquid chromatograph with a Zorbax SB-C18 (9.4 mm × 250 mm) column. Fractions were monitored by TLC, and compounds were visualized by UV light (254 nm and 365 nm) and by heating the silica gel plates after spraying with 10% H_2SO_4 in EtOH. All solvents used in CC were distilled, including light petroleum (60–90 °C).

2.2. Plant material

The stems of *K. heteroclita* were collected from Xishuangbanna Autonomous Prefecture, Yunnan Province, People's Republic of China, in 2015 and identified by Prof. Heng Li, Kunming Institute of Botany. A voucher specimen (KIB 2015102101) has been deposited in our laboratory.

2.3. Extraction and isolation

The air-dried and powdered stems of *K. heteroclita* (8 kg) were extracted four times with 70% aqueous acetone (4 × 25 L, 3 days each) at room temperature and filtered to yield a filtrate, which was then evaporated under reduced pressure and partitioned successively with EtOAc and *n*-BuOH, respectively. The EtOAc soluble portion (261 g) was subjected to column chromatography (CC) on silica gel (1.5 kg, 80–100 mesh), eluted with a $CHCl_3$-Me_2CO gradient system (1:0–0:1), five fractions (Fr.A–Fr.E) were therefore obtained based on TLC analysis. Fraction B (51 g) was chromatographed on RP-C_{18} silica gel CC (50–100% MeOH/H_2O) to yield five main fractions (Fr.B1–Fr.B5). Fr.B2 (3 g) was subjected to repeated CC over silica gel (10 g, 100–200 mesh), eluted with petroleum ether-acetone (gradient system: 40:1–1:1), to yield compound **15** (14.33 mg). Compounds **1** (18.17 mg) and **7** (4.02 mg) was crystallized from fraction B3 and then further purified by semipreparative HPLC (70% MeCN-H_2O). The further separation of Fr.B3 (11.5 g) by silica gel CC, eluted with gradient petroleum ether-acetone (40:1–1:1), yielded mixtures Fr.B3.1–Fr.B3.6. Fr.B3.1 (2.0 g) was further purified by medium-pressure CC on RP-C_{18} (MeOH-H_2O gradient, 40%–100%), followed by semipreparative HPLC (70% MeCN-H_2O), to afford compound **16** (12.01 mg). Fr.B3.2 (2.4 g), Fr.B3.3 (4.0 g), and Fr.B3.4 (2.1 g) were respectively separated by medium-pressure CC on RP-C_{18} (MeOH-H_2O gradient, 40%–100%), and then purified by semipreparative HPLC (50%–70% MeCN-H_2O). After the two separate steps, Fr.B3.2 gave compounds **8** (32.66 mg) and **9**