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A high-performance liquid chromatography with circular dichroism detector for determination of stereochemistry of 6, 9-oxygen bridge dibenzocyclooctadiene lignans from *kadsura coccinea*

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[ABSTRACT] The stereochemistry of two 6, 9-oxygen bridge dibenzocyclooctadiene lignans from *Kadsura coccinea*, are difficult to separate and very unstable. The present study was designed to develop a high-performance liquid chromatography using circular dichroism detection for the analysis of the stereochemistry. A new 6, 9-oxygen bridge dibenzocyclooctadiene lignans named Kadsulignan Q was firstly found with an *S*-biphenyl configuration. The other compound was identified as Kadsulignan L with an R- biphenyl configuration. In order to obtain kinetic data on their reversible interconversion, the stability was measured at different deuterated solvents such as deuterated methanol, deuterated chloroform and deuterated dimethylsulfoxide. The lignans were more unstable and converted more easily in deuterated methanol than in deuterated chloroform and deuterated dimethylsulfoxide.

[KEY WORDS] 6, 9-Oxygen bridge dibenzocyclooctadiene lignans; *Kadsura coccinea*; High-performance liquid chromatography using circular dichroism detection (HPLC-CD); Kadsulignan Q; Kadsulignan L

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Introduction

Kadsura coccinea is a climbing plant andwidely distributed in the southern part of China. The dried roots and stems are used for the treatment of rheumatoid arthritis, gastric and duodenal ulcers in folk medicine [1]. The major active constituents are dibenzocyclooctadiene lignans and triterpenoids [2-3].

6, 9-oxygen bridge dibenzocyclooctadiene lignans are a kind of uncommon lignans; only eight compounds with same skeleton have been reported, including synthetic 6, 9-dibenzocyclooctadiene and other compounds separated from *Kadsura coccinea* and *Clerodendron inermethe* [4-8]. One of these compounds exhibited a significant activity against HIV *in vivo* [6]. In addition, most of 6, 9-oxygen bridge dibenzocyclooctadiene lignans are separated from the *Kadsura coccinea*. Kadsulignan L. is one of the 6, 9-oxygen bridge dibenzocyclooctadiene lignans. In the previous study [6], Kadsulignan L has been isolated and identified by NMR spectrum. However, two compounds are found to coexist by the HPLC-UV spectrum from the sample of Kadsulignan L after a period of time. After further separation by semi-preparative liquid chromatography, there are still two compounds in the isolated sample. Moreover, the NMR data of isolated compounds were same as compared to Kadsuligan L.[6]. Therefore, two compounds may be isomeride. To prove this hypothesis, a high-performance liquid chromatography using circular dichroism detection (HPLC-CD) was developed in the present study.

The stereochemistry of natural products detected by HPLC-CD had been reported before [9-10]. Non-chiral HPLC-CD has been used for not only drug purity analysis and monitoring enantioselective syntheses, but also characterization of enantioselective separations [11]. The stereochemistry of Kadsulignan L has been extensively discussed in the previous reports [6, 8] (Fig. 1). Its stereochemistry is determined as Compound **1** which has an *S*-biphenyl configuration in the

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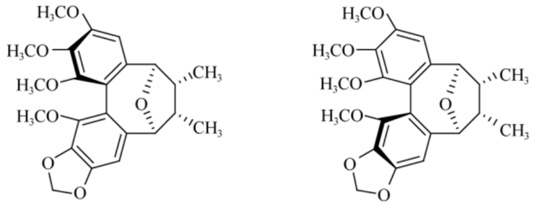
first report, but then revised to Compound **2** which has an *R*-biphenyl configuration by the degradation reaction [6]. Both of Compounds **1** and **2** have the same CD spectra, according to the reports. In the present study, an HPLC-CD on line analysis was used to determine the configuration of 6, 9-oxygen bridge dibenzocyclooctadiene lignans for the first time. And a kinetic study on their reversible interconversion was also discussed. The results might provide useful information for the determination of stereochemistry of natural products, especially the isomeride.



1

2

Fig. 1 Structures of Compounds 1 and 2

Materials and Methods

Instruments

An Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) comprising of a quaternary solvent delivery system, two pumps, a column temperature controller, a sample injector and a circular dichroism detector or diode array detector coupled to an analytical workstation was used to acquire chromatograms. UHPLC-ESI-MS data were obtained using a Waters UPLC-Primer XE and Thermo Hypersil Syneronis (ACQUITY UPLC M-Class, made in America). ^{1}H and ^{13}C NMR spectra were obtained on a JEOL-600MHz-NMR system (Bruker DRX 600 MHz NMR Spetrometer, made in German).

Reagents and chemicals

6, 9-oxygen bridge dibenzocyclooctadiene lignans were separated from the *K. coccinea*, the dried rhizoma of *Kadsura coccinea* were collected in Guangxi Province, Peoples Republic of China (identified by forestry institute in Jianhe County, Guizhou Province, China). The solvents for the preparation of the mobile phases were HPLC-grade.

HPLC-DAD experiment

5 mg of powdered 6, 9-oxygen bridge dibenzocyclooctadiene lignin was dissolved in 200 μL of methanol in a sample bottle. The analyses were performed using a Cosmosil Packed Column C_{18}-MS-II (4.6 mm × 250 mm, 5 μm, i.d.) (made in Japan). The column temperature was maintained at 25 °C. The mobile phase was consisted of acetonitrile (A) and water (B) and the gradient program was 55%-62.5% (A) in 0–40 min. The flow rate was 1.0 mL·min^{-1}. The sample injection volume was 20 μL. Detection wavelength was set at 210 nm.

Characterization

The same molecular formula $C_{23}H_{26}O_7$ for the two compounds was clarified from UHPLC-ESI-MS. NMR spectroscopy was used to complete the structural characterization. The ^{1}H NMR and ^{13}C NMR data of Compounds **1** and **2** matched those reported in the literature [12].

HPLC-CD experiment

The conditions of HPLC-CD were the same as HPLC-AD, except for the use of CD detector at 210 nm.

Stability analysis experiment

5 mg samples were dissolved in deuterated methanol, deuterated chloroform and deuterated dimethylulfoxide, respectively. ^{1}H NMR spectra were determined at 2, 24, 72, and 144 h.

Results and Discussion

HPLC-DAD analysis

Compounds **1** and **2** were analyzed on an Agilent 1200 HPLC system under the chromatographic conditions and the chromatograms showed two peaks and the same ultraviolet spectrum was also observed (Fig. 2). Moreover, the final

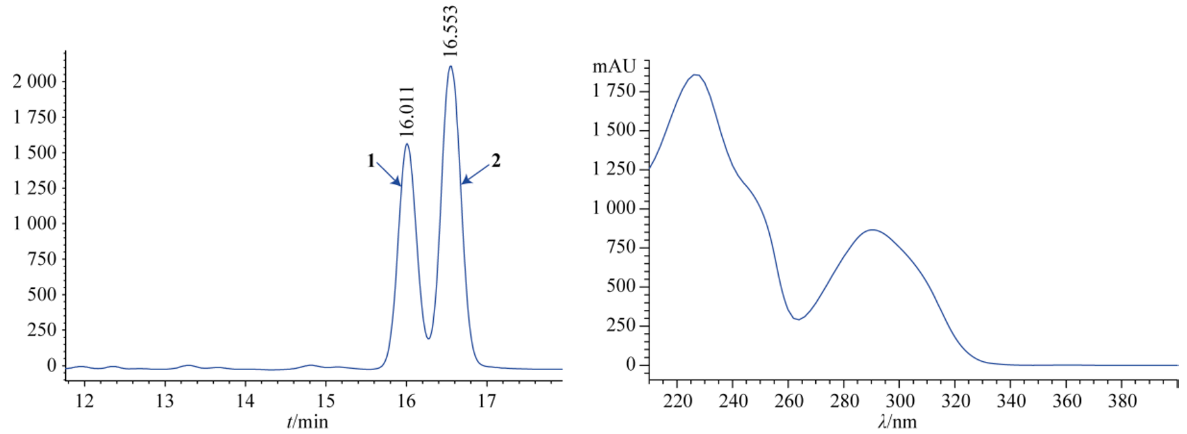


Fig. 2 Chromatogram of Compounds 1 and 2 at 210 nm and the Ultraviolet spectra