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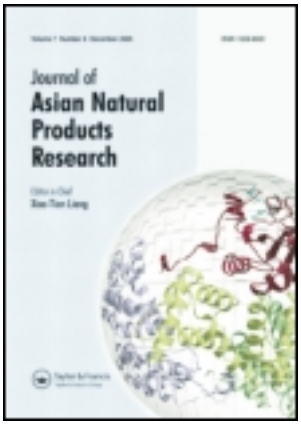

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Determination of atropisomeric configurations of macrocyclic bisbibenzyls by HPLC-CD/UV and quantum chemical calculations

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Determination of atropisomeric configurations of macrocyclic bisbibenzyls by HPLC-CD/UV and quantum chemical calculations

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Isoriccardin C (**1**) and riccardin D (**2**), isolated from the liverwort *Reboulia hemisphaerica*, were first characterized to be a mixture of two enantiomeric atropisomers by online chiral high-performance liquid chromatography-circular dichroism (HPLC-CD) analysis. Exemplarily for bisbibenzyls of the diarylether-biphenyl type, the absolute atropisomeric configurations of compunds **1** and **2** were determined by the analysis of their CD data coupled with quantum chemical CD calculations.

Keywords: *Reboulia hemisphaerica*; atropisomer; bisbibenzyls; online HPLC-CD; quantum chemical CD calculations

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1. Introduction

Macrocyclic bisbibenzyls are unique metabolites detected mainly in liverwort species and found to have antifungal, anticancer, antioxidant, antiplatelet aggregation, antibacterial, and antiviral activities [1–4]. Their structures can be subdivided into four groups according to their linkage types when the two bibenzyl units are oxidatively coupled, which are A-C, B-D (biphenyl-biphenyl type, I), A-C, B-O-D (biphenyl-diaryl ether type, II), A-O-C, B-D (diaryl ether-biphenyl type, III), and A-O-C, B-O-D (diarylether-diaryl ether type, IV), respectively (Scheme 1) [5–7].

The existence of atropisomerism of type I bisbibenzyls, such as isoplagiochins C, D, and of type II-linked derivatives of isoplagiochin B and dihydroisoplagiochin B, has been reported in liverworts, due to the hindered rotation of two benzene rings around the single bond by the *ortho*-substituents on the biphenyl parts [8–10]. In ongoing studies on the biologically active bisbibenzyls from liverworts [2,11,12], we found that the type III structures, when *ortho*-hydroxyl(s) occurred at the biphenyl linkage, were also optically active. Here, we first report the characterization and assignment of the absolute configurations of two macrocyclic bisbibenzyls isoriccardin C (**1**) and riccardin D (**2**) (Figure 1) [13,14], isolated from *Reboulia hemisphaerica* which is a folk medicine traditionally used in China [15], by high-performance liquid chromatography-circular dichroism (HPLC-UV/CD) technique and quantum chemical CD calculations.

2. Results and discussion

The Et_2O extract of the liverwort *R. hemisphaerica* was chromatographed on silica gel and the resulting fractions were purified by Sephadex LH-20 and HPLC to get nine known bisbibenzyl

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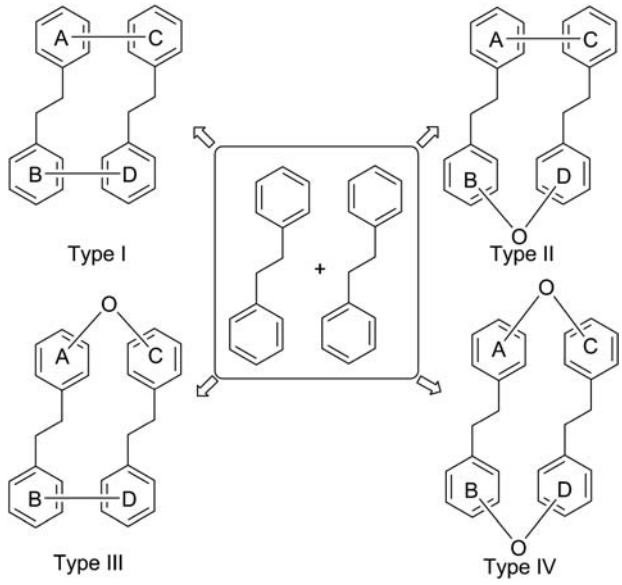

Scheme 1. Four types (I–IV) of cyclic bisbibenzyls from liverworts.

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derivatives. Among them, the type III bisbibenzyls, isoriccardin C (**1**) and riccadin D (**2**), with the same linkage units but different linkage position, were found to be optically active at room temperature, although not equipped with any of the traditional stereogenic elements that rendered molecules conformationally stable *per se*. Compound **1** was found to be optically active with levorotation ($[\alpha]_D^{20}$ -2.2, $c = 0.3$, acetone), and showed a weak CD spectrum with a negative Cotton effect at *ca.* 220 nm and a positive Cotton effect at *ca.* 260 nm and 280 nm, while compound **2** was also levorotatory ($[\alpha]_D^{20}$ -3.9, $c = 0.3$, acetone) and showed a weak CD spectrum with a negative Cotton effect at *ca.* 220 nm and a positive Cotton effect at *ca.* 270 nm, respectively.

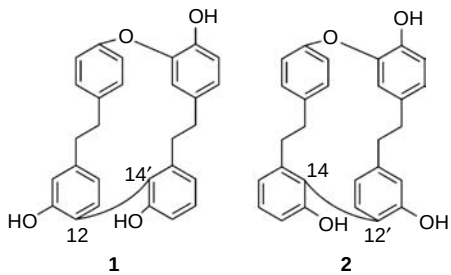

Figure 1. Structures of compounds **1** and **2**.

The respective atropisomers of compounds **1** (corresponding to peaks 1a and 1b) and **2** (corresponding to peaks 2a and 2b) were determined by chiral HPLC (Chiralpak AS-H) at 220 nm (Figure 2(a)). For further stereochemical analysis of compounds **1** and **2**, the direct stereochemical online analysis of the peaks by HPLC-CD coupling was used. As shown in Figure 2(b), these HPLC-UV peaks corresponding to the respective atropisomers were demonstrated by their opposite CD effects. Compound **1** gave a positive HPLC-CD signal for peak 1a ('faster') and a negative one for peak 1b ('slower'), while compound **2** gave a negative HPLC-CD signal for peak 2a ('faster') and a positive one for peak 2b ('slower') in the reverse order of compound **1**.

The online CD spectra of compounds **1** and **2** provided two pairs of mirror-imaged CD curves (Figure 2(d) and (c)). However, a merely empirical interpretation of the CD spectra was impossible for the assignment

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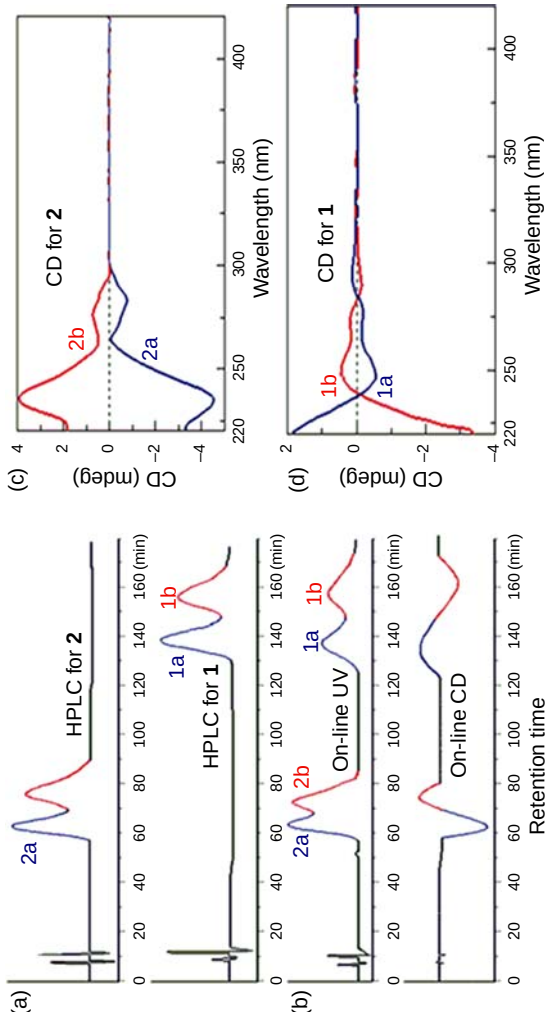

Figure 2. (a) Resolution of the atropisomeric components, riccardin D (**2**) and isoriccardin C (**1**) from *R. hemisphaerica* on Chiralpak AS-H. Mobile phase: *n*-hexane–2-propanol (80:20, v/v), flow rate: 0.5 ml/min, UV wavelength: 220 nm. (b) In the HPLC-UV-CD, UV (upper) and CD (lower) chromatograms of **2** and **1** detected at 220 nm. Column: Chiralpak AS-H. Mobile phase: *n*-hexane–2-propanol (80:20, v/v), flow rate: 0.5 ml/min, UV wavelength: 220 nm. (c) The measurement of full online CD spectra for **2**. (d) The measurement of full online CD spectra for **1**. Column: Chiralpak AS-H. Mobile phase: *n*-hexane–2-propanol (80:20, v/v), flow rate: 0.5 ml/min, UV wavelength: 220 nm.

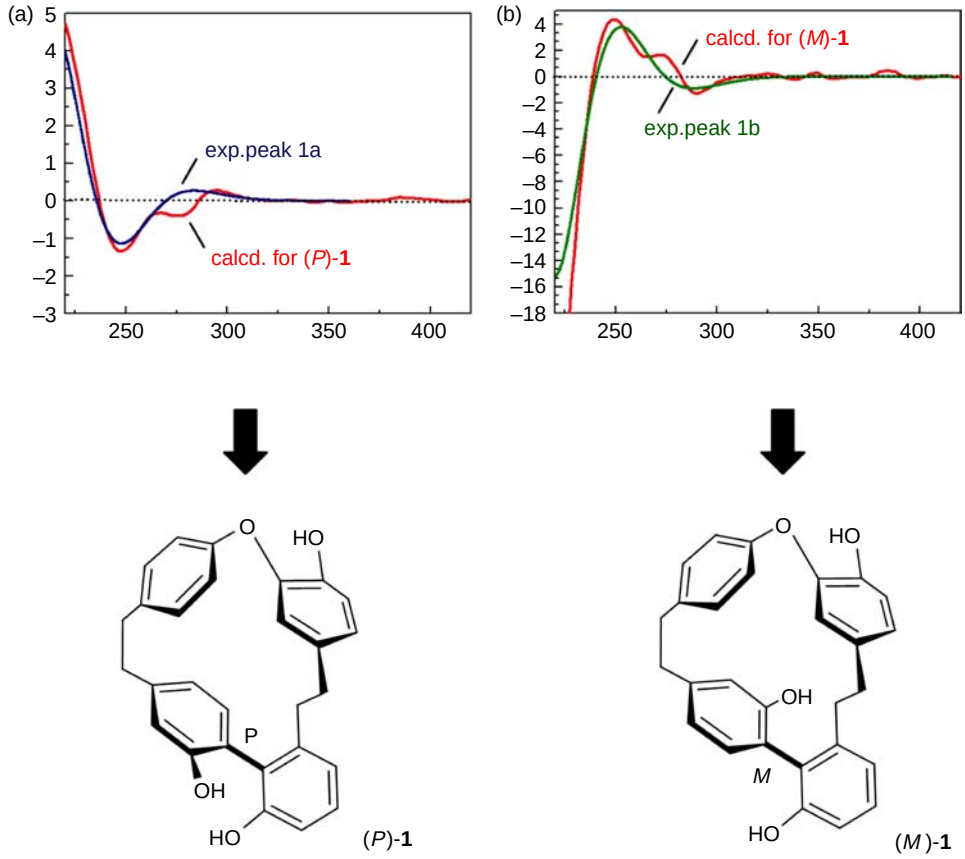

Figure 3. Stereochemical assignment of the two atropisomers of isoriccardin C (**1**), by LC-CD coupling and quantum chemical CD calculations.

of the absolute configurations of the respective atropisomers of compounds **1** and **2**. Quantum chemical CD calculation was employed here to determine their absolute configurations. As already shown in the case of the related bisbibenzyl isoplagiochin C [8,9], the rotational barriers for the biphenyl axis of isoriccardin C (**1**) and riccardin D (**2**) (joining C-12 and C-14′, or C-14 and C-12′, see Figure 1) were calculated as 30.86 and 28.19 kcal mol^{-1} (see Figures S1 and S2 in the Supporting Information), respectively, revealing that the biphenyl axis was configurationally more stable at room temperature as compared to the diarylether units. Therefore, arbitrarily starting with the *M*-configured stable biaryl axis of compounds **1** and **2**, the geometries of the stable conformations found with semiempirical AM1 method were further optimized using density functional theory (DFT) (B3LYP/6-31G*). For these, CD calculations by means of time-dependent DFT (TDDFT) were carried out. All single CD spectra were then added up following the Boltzmann statistics to give the theoretical overall CD spectrum. *P*-configured compounds **1** and **2** were obtained by the reflection of the spectrum of (*M*)-**1** and (*M*)-**2** on the wavelength axis. The predicted overall CD curves thus obtained were UV-corrected and compared with the experimental spectra of the 'faster' (peaks 1a and 2a) and the 'slower' (peaks 1b and 2b) eluted atropisomers of compounds **1**

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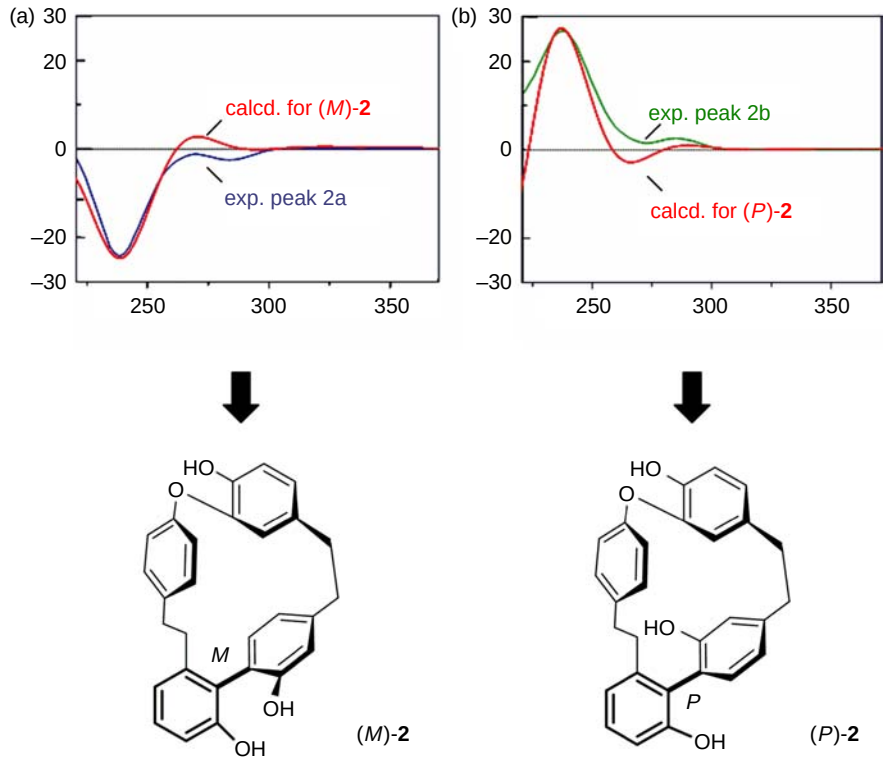

Figure 4. Stereochemical assignment of the two atropisomers of riccardin D (**2**), by LC-CD coupling and quantum chemical CD calculations.

and **2**. The comparison revealed quite good agreements between (*P*)-**1** and peak 1a (Figure 3(a), left), (*M*)-**1** and peak 1b (Figure 3(b), right), (*M*)-**2** and peak 2a (Figure 4(a), left), as well as (*P*)-**2** and peak 2b (Figure 4(b), right), thus permitting assignments of the absolute configurations to the corresponding atropisomers. In addition, the weak optically active properties of the above two compounds at room temperature indicated that the atropisomers were unequally populated. The same phenomena are also found with other macrocyclic bisbibenzyls [8,9].

3. Experimental

3.1 General experimental procedures

The HPLC-CD coupling system consisted of a Jasco pump PU 2089 with Rheodyne 20 μl sample loops, a CD 2095 detector and Borwin chromatographic software (Jasco, Tokyo, Japan). Offline CD spectra were recorded with a Chirascan circular dichroism spectrometer. The HPLC equipment used for the isolation of atropisomers was Agilent 1100 series system and Agilent HPLC workstation (Agilent, Palo Alto, CA, USA). IR spectra were recorded on a Thermo Nicolet NEXUS 670 FT-IR Spectrophotometer (Nicolet, Madison, WI, USA) with KBr disks. NMR spectra were measured on a Bruker Avance DRX-600 spectrometer (Bruker, Zurich, Switzerland) operated at 600 (^{1}H) or 150 (^{13}C) MHz, in chloroform-*d*, δ in ppm rel. to Me_4Si as an internal standard. 2D NMR spectra were recorded with standard pulse programs and acquisition parameters. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was carried out on API 4000 triple-stage

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quadrupole instrument. HR-EI-MS was performed on VG ZAB-2F mass spectrometer, in *m/z* (rel. %). Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Plant, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography (CC). Methanol, *n*-hexane, and 2-propanol used for HPLC were of chromatographic grade (Siyou Chemical Factory, Tianjin, China). All other chemicals used in the study were of analytical grade (Laiyang Chemical Factory, Shandong, China).

3.2 Plant materials

R. hemisphaerica was collected from Guizhou Province of China and authenticated by Professor Yuan-Xin Xiong (School of Agricultural Sciences, Guizhou University, China). A voucher specimen has been deposited in the School of Pharmaceutical Sciences, Shandong University (accession number: No. TX-18-200807-RH).

3.3 Isolation of isoriccardin C and riccardin D

The air-dried aerial parts of *R. hemisphaerica* (380 g) were extracted exhaustively with 95% ethanol (3 liters) by heating until reflux began, and maintained for 2.5 h. The process was repeated three times and the combined extract evaporated to dryness *in vacuo* yielded 32 g crude extract. The crude extract was suspended in H_2O (200 ml) and partitioned sequentially with Et_2O (250 ml) and *n*-BuOH (250 ml) for three times. The diethyl ether extract (12.7 g) was fractionated by silica gel CC using a gradient of petroleum ether in acetone to yield 390 fractions. Each fraction (150 ml) was monitored by silica gel TLC, visualized under UV lights (254 and 365 nm) and by spraying with 10% H_2SO_4 in ethanol. Fractions with similar TLC patterns were combined and evaporated to yield 13 fractions (Frs 1–13). Separation of Fr. 11 (1.7 g) by semi-preparative HPLC (MeOH–H_2O 64:36, reversed-phase ZORBAX SB-C_{18} column, 250 mm × 9.4 mm, I.D., 5 μm) afforded nine known bisbibenzyls including isoriccardin C (**1**) and riccardin D (**2**).

3.4 HPLC-CD analysis

The chiral stationary phase was Chiralpak AS-H (250 mm × 4.6 mm, Daicel, Tokyo, Japan). The mobile phase was a mixture of 2-propanol–*n*-hexane (20:80, v/v). The flow rate was 0.5 ml/min at room temperature. UV and CD detectors were set at 220 nm. The injection volume was 1 μl.

3.5 Computational methods

Conformational analysis of compounds **1** and **2** was performed by means of the semiempirical AM1 method (a semiempirical method for the quantum calculation of molecular electronic structure in computational chemistry) [16] in order to find all minimum geometries that are significantly populated at ambient temperature and further optimized through a DFT approach B3LYP/6-31G* (a quantum mechanical modeling method used in physics and chemistry to investigate the electronic structure (principally the ground state) of many-body systems, in particular atoms, molecules, and the condensed phases) [17,18] as implemented in the program package GAUSSIAN 03, starting from the corresponding *M*-configuration structures. The geometries of the stable conformations obtained were further optimized at the B3LYP/6-31G* level, followed by calculations of their harmonic vibration frequencies to verify their stabilities and thence calculations of room-temperature free energies.

Electronic excitation energies and rotational strengths were calculated using TDDFT at the same level in the gas phase both in velocity and length formalism for

the first 30 states. The rotatory strengths were summed and energetically weighted following the Boltzmann statistics and simulated into an ECD curve using the Gaussian function:

$$\Delta\varepsilon(E) = \frac{1}{2.297\times10^{-39}}\frac{1}{\sqrt{2\pi\sigma}} \times \sum_i \Delta E_{0i} R_{0i}\, \mathrm{e}^{-[(E-\Delta E_{0i})/2\sigma]^2},$$

where σ ($\sigma = 0.1$ eV and R_{vel}) is the width of the band at $1/e$ height, whereas ΔE_i and R_i are the excitation energies and rotatory strengths for transition, respectively.

To calculate the possible rotational barriers of A and B, the respective transition structures were located using the synchronous transit-guided quasi-Newton method [19] starting from a global minimum geometry. A frequency analysis was performed to confirm these first-order saddle points. The intrinsic reaction coordinate pathways from the transition states to two local minima were traced in order to verify that each saddle point linked the two desired minima.

The molecular dynamics simulations were carried out using the COMPASS Force Field [20] as implemented in the Materials Studio software (version 4.0; Accerlys Software Inc.). All simulations were performed in the NVT ensemble with a time step of 1 fs. The temperature was controlled using the Andersen thermostat [21] with collision ratio of 1.0. The total simulation time was 500 ps, and the simulation results were analyzed from the coordinates which were saved every 5000 steps.

3.6 Identification of bisbibenzyls

The structural identification of bisbibenzyls was carried out by IR, MS, 1D NMR, and 2D NMR spectra.

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