Heteroclitins R-S: new dibenzocylooctadiene lignans from *Kadsura heteroclita*

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**[ABSTRACT]**

**AIM:** To study the dibenzocylooctadiene lignans from the stems of *Kadsura heteroclita*.

**METHOD:** Chromatographic separations of silica gel and semi-preparative HPLC were used. All of the structures were elucidated on the basis of spectroscopic analysis, including 2D-NMR and HR-MS techniques.

**RESULTS:** Four dibenzocylooctadiene lignans were isolated from *K. heteroclita*. Their structures were identified as heteroclitin R (1), heteroclitin S (2), gonisin O (3), and schisanlignone A (4).

**CONCLUSION:** Heteroclitin R (1) and heteroclitin S (2) are new natural lignans.

**[KEY WORDS]** Dibenzocylooctadiene Lignan; *Kadsura heteroclita*; Schisandraceae; Heteroclitins R-S

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**Introduction**

The stems of *Kadsura heteroclita* (Roxb.) Craib (Schisandraceae) are commonly used in Chinese traditional medicine to promote vital energy and blood circulation, to expel wind-evil, and to remove wetness-evil [1]. The plants are known to be a rich source of lignans and triprenoids, which have been found to possess various beneficial pharmacological effects [2-7]. In previous studies from this laboratory, some dibenzocyclooctadiene lignans were isolated from *K. heteroclita* [¹, 8]. Efforts have continued to search for bioactive natural products from *K. heteroclita* indigenous to the southern area of China. Repeated column chromatography of the Et₂O extract of the stems of *K. heteroclita* led to the isolation and identification of a new dibenzocylooctadiene lignan named heteroclitin R (1), a new natural product named heteroclitin S (2), and two known compounds, gonisin O (3) [⁹] and schisanlignone A (4) [¹⁰]. Compounds 3 and 4 were isolated from *K. heteroclita* for the first time. Their structures and stereochemistries were elucidated by spectroscopic methods.

![Fig. 1 Structures of compounds 1 and 2](image)

**Results and Discussion**

Compound 1, obtained as a yellow amorphous powder, was assigned the molecular formula C₂₇H₂₈O₉ by HR-EI-MS (m/z 519.163 [M + Na]⁺, Calcd. 519.163 1). The ¹H NMR
and $^{13}$C NMR data indicated that 1 is a dibenzocyclooctadiene lignan. The characteristic proton signals at $\delta_H$ 4.69 and 4.16 (2H, d, $J = 8.8$ Hz), and a quaternary carbon signal at $\delta_C$ 78.3, indicated that 1 possessed a spiroene ring, similar to heteroclitin H [8].

The $^1$H NMR spectrum of 1 (Table 1) showed the presence of two H-atoms at $\delta_H$ 7.38 (1H, s) and 6.53 (1H, s), which were assignable to H-4 and H-11, respectively. The $^1$H NMR spectrum also showed two doublet methyl groups at $\delta_H$ 1.03 (3H, d, $J = 6.6$ Hz) and 0.94 (3H, d, $J = 7.3$ Hz), indicating the presence of two secondary methyl groups on the cyclooctadiene ring. Two methoxy groups ($\delta_H$ 4.02, 3.78, each 3H, $s$), one methylenedioxy group ($\delta_H$ 6.07, 6.02, each 1H, d, $J = 1.5$ Hz), and two methine protons at $\delta_H$ 3.14 and 2.01 (each 1H, $m$) also be observed in the $^1$H NMR spectrum.

The cross peaks of H-4 ($\delta_H$ 7.38) with $\delta_C$ 135.7 and 153.2, and of H-11 ($\delta_H$ 6.53) with $\delta_C$ 130.6 and 150.8 in the HMBC spectrum suggested that these four carbons were C-3, C-2, C-12, and C-13, respectively (Fig. 2). Two H-atoms of a O-bearing CH$_2$ group resonating at $\delta_H$ 4.69 and 4.16 correlated with quaternary C-atoms at 143.5 (C-14) and 141.2 (C-5), and the carbonyl group at $\delta_C$ 193.8 in HMBC suggesting that I contained an $\alpha$, $\beta$, $\gamma$, $\delta$-diene structure, and a carbonyl group at C-1, similar to kadsutherin C [10]. The HMBC correlations of $\delta_H$ 6.07 and 6.02 with $\delta_C$ 130.6 and 150.8, and $\delta_H$ 4.02 (3H, $s$, with $\delta_C$ 153.2, and $\delta_H$ 3.78 (3H, $s$, with $\delta_C$ 135.7, suggested the substitution of the two methylenedioxy groups at C-12 and C-13, and that the two methoxyl groups were located in C-3 and C-2, respectively.

![Fig. 2 Key HMBC and ROESY correlations of 1](image-url)

The signal at $\delta_H$ 7.38 (H-4) was shifted downfield by ca. 1 ppm as compared with “common” aromatic H-atoms, and showed HMBC correlation (Fig. 2) with the carbonyl group at $\delta_C$ 198.3, indicating that the carbonyl group was located at C-6 and was conjugated with the aromatic ring [12]. The methyl protons at $\delta_H$ 1.03 correlated with $\delta_C$ 198.3 in the HMBC suggesting that the methyl group was assignable to C-17.

The proton signals at $\delta_H$ 5.78 (1H, $q$, $J = 7.3$ Hz), 1.70 (3H, dd, $J = 7.3, 1.8$ Hz), and 1.62 (3H, d, $J = 1.8$ Hz), and the carbon signals at $\delta_C$ 167.1, 127.2, 137.1, 15.6, and 20.3 showed the presence of a tigloyl group. HMBC correlations of H-11 with $\delta_C$ 78.4 (C-9), and $\delta_H$ 5.70 (1H, d, $J = 5.1$ Hz, H-9) with the C-1' ($\delta_C$ 167.1, C-7 ($\delta_C$ 43.7) and C-8 ($\delta_C$ 44.3) and C-17 ($\delta_C$ 10.9) in the HMBC spectrum, indicated an ester group substituted at C-9.

The CD spectrum of 1 with negative Cotton effect at 239 nm and a positive Cotton effect at 223 nm indicated 1 had a $\delta$-biphenyl configuration [13]. The ketone group (C=O) at C-6 indicated a twist-boat (TB) conformation as the only possible conformation for the cyclooctadiene ring [14-15]. NOESY correlations of H-4/CH$_2$O-3, H-4/CH$_2$-5', H-7/H-8, H-7/CH$_2$-17, H-8/CH$_2$-18, CH$_2$-18/CH$_2$-17, H-9/H-8, H-9/H-11, and H-9/CH$_2$-5' strengthened the substituent positions and the stereochemical assignments (Fig. 2). Thus, 1 was elucidated as shown in Fig. 1, and as a new compound, was named heteroclitin R.

Compound 2, obtained as a colorless powder, has the molecular formula C$_2$H$_2$O$_5$, based on HR-ESI-MS data (m/z 423.1417 [M + Na]$^+$, Calcd. 423.1420). The $^1$H- and $^{13}$C-NMR spectra (Table 1) indicated that 2 is a dibenzocyclooctene lignan.

The $^1$H NMR spectrum of 2 showed the presence of two aromatic protons at $\delta_H$ 7.49 and 6.38 (each 1H, $s$), assignable to H-4 and H-11, respectively. The resonances at $\delta_H$ 1.00 and 0.82 (each 3H, d, $J = 6.6$ Hz) could be assigned to the cis-oriented CH$_2$-17 and CH$_2$-18 [15]. The $^1$H NMR spectrum also showed three methoxyl groups at $\delta_H$ 3.94, 3.92, and 3.81 (each 3H, $s$), a methylenedioxy group at $\delta_H$ 6.06, 6.03 (each 1H, d, $J = 1.5$ Hz), two methylene protons at $\delta_H$ 2.63 and 2.24 (each 1H, $m$), and two methine protons at $\delta_H$ 2.63 and 1.79 (each 1H, $m$).

The correlations of H-4 with $\delta_C$ 141.1 and 148.9, and of H-11 with $\delta_C$ 152.2, 133.6, and 147.5 in the HMBC spectrum (Fig. 3), suggested that these five carbons were located at C-2, C-3, C-12, C-13, and C-14, respectively. The correlations of the methylenedioxy protons ($\delta_H$ 6.06, 6.03) with $\delta_C$ 141.1 (C-2) and 148.9 (C-3) in the HMBC, indicated the methylenedioxy group was connected between C-2 and C-3. The cross peaks of the methoxy groups at $\delta_H$ 3.81, 3.92, and 3.94 (each 3H, $s$) with the carbons at $\delta_C$ 140.9, 152.2, and 133.6, respectively, revealed these three methoxy groups were located at C-1, C-12, and C-13. The absence of a typical carbon signal for the methylenedioxy group at $\delta_C$ 100-102 in the $^{13}$C NMR spectrum (Table 1) and the presence of one proton signals at $\delta_H$ 5.89 (1H, br s) without any correlation in HMBC spectrum, suggested the presence of hydroxyls on the aromatic rings [16]. Cross peaks of $\delta_H$ 5.89 (-OH) with $\delta_C$ 147.5 (C-14), 133.6 (C-13), and 115.4 (C-15) indicated that the hydroxy was located at C-14. The $^{13}$C NMR spectrum at $\delta_C$ 200.3 revealed the presence of a conjugated ketone moiety. HMBC cross peaks of H-4 and the methyl group at $\delta_H$ 1.00 with $\delta_C$ 200.3, indicated that the C=O group was located at C-6, similar to compound 1.
The CD spectrum of 2 with a negative Cotton effect at 228, 280 nm and a positive Cotton effect at 247 nm indicated that 2 possessed an R-biphenyl configuration. NOESY correlations of H-11 with CH3-12, H-8β with CH2-12, CH2-18 with CH2-17, CH2-18 with H-9α, H-8β with H-11, H-9β with H-11, and CH3O-13 with OH-14 strengthened the substituent positions and the stereochemical assignments (Fig. 3). Thus, the structure of 2 was determined as shown in Fig. 1. The structure of 2 was the same as the synthetic compound 2a, and is a new natural product, named as heterolin S (Fig. 1).

Compounds 3 and 4 were identified as gomisin O and schislanignone A by comparison their UV, IR, EI-MS, 1H NMR, and 13C NMR data with those reported and assignments (Fig. 3). Thus, the structure of 2 was the same as the synthetic compound 2a, and is a new natural product, named as heterolin S (Fig. 1).

**Experimental**

**General**

The IR spectra were recorded as KBr pellets on the Avatar 360E.S.P spectrophotometer (Thermo Nicolet Co.). The UV spectra were measured on a Shimadzu UV-260 spectrophotometer for HR-EI-MS. 1H NMR and 13C NMR spectra were measured with a JASCO J-715 spectropolarimeter. The CD spectrum of 2 was obtained as a yellow amorphous powder; [α]D +7.9° (c 0.29 MeOH); UV (MeOH) λmax (log ε): 200 nm (4.78); IR (KBr) νmax: 2 953, 1 715, 1 651, 1 117 cm⁻¹; 1H NMR and 13C NMR, see Table 1; HR-ESI-MS: C27H28O9 [M + Na]⁺, Calcd. 519.163 3 [M + Na]⁺, Caled. 519.163 1); CD (c 0.29, MeOH), (θ)D (nm): +22477 (223), −16746 (239).

| Table 1 1H NMR and 13C NMR spectra for compounds 1 and 2 (400 MHz and 100 MHz, CDCl3) |
|---|---|---|---|
| No. | ΔδH | ΔδC | δH | δC |
| 1 | 193.8 | 140.9 |
| 2 | 153.2 | 141.1 |
| 3 | 135.7 | 148.9 |
| 4 | 127.3 | 7.38 | 104.1 | 7.49 |
| 5 | 141.2 | 134.4 |
| 6 | 198.3 | 200.3 |
| 7 | 43.7 | 3.14 | 44.6 | 2.63 |
| 8 | 44.3 | 2.01 | 41.2 | 1.79 |
| 9 | 78.4 | 5.70 | 40.1 | 2.63, 2.24 |
| 10 | 128.7 | 136.1 |
| 11 | 101.2 | 6.53 | 102.8 | 6.38 |
| 12 | 130.6 | 152.2 |
| 13 | 150.8 | 133.6 |
| 14 | 143.5 | 147.5 |
| 15 | 120.5 | 115.4 |

**Plant material**

The stems of *Kadsura heteroclita* were collected in Fengqing County, Yunnan Province, China in July of 1997, identified by CHEN Dao-feng. A voucher specimen (DFC-JXT9707) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, China.

**Extraction and Isolation**

The stems (9 kg) of *K. heteroclita* were air-dried, ground and extracted five times with 95% ethanol (5 × 40 L) at room temperature and filtered. The alcoholic extract was evaporated in vacuo to yield a semisolid (1 200 g). The semisolid was suspended in water (2000 mL) and extracted seven times with diethyl ether (7 × 3 L). This ether solution was concentrated to yield a residue (75 g). The residue was chromatographed on silica gel (1 500 g, 200–300 mesh), employing petroleum ether (60–90 °C) containing increasing amounts of ethyl acetate as eluent (100% petroleum ether, 98 : 2, 95 : 5, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 100% ethyl acetate) to afford nine fraction. Fraction 6 [petroleum ether–EtOAc (7 : 3), 12.5 g] was subjected to repeated silica gel column chromatography with petroleum ether-acetone (8 : 1 : 3 : 1). Fr. 6-3 (7.2 mg) was purified with semi-preparative HPLC (65% MeOH–H2O, flow rate 2 mL·min⁻¹) to yield 1 (7 mg) and 2 (8.5 mg). Fraction 5 [petroleum ether–EtOAc (8 : 2), 10.3 g] was also subjected to repeated silica gel column chromatography with petroleum ether–acetone (8 : 1). Semi-preparative HPLC (70% MeOH–H2O, flow rate 2 mL·min⁻¹) of fraction 5-4 (112 mg) gave 3 (12 mg) and 4 (5 mg).

**Structure identification**

Heterocalitin R (1) obtained as a yellow amorphous powder; [α]D +7.9° (c 0.29 MeOH); UV (MeOH) λmax (log ε): 200 nm (4.78); IR (KBr) νmax: 2 953, 1 715, 1 651, 1 117 cm⁻¹; 1H NMR and 13C NMR, see Table 1; HR-ESI-MS: C27H28O9 [M + Na]⁺, Calcd. 519.163 3 [M + Na]⁺, Caled. 519.163 1); CD (c 0.29, MeOH), (θ)D (nm): +22477 (223), −16746 (239).
Heteroclitin S (2) obtained as a colorless amorphous powder; [α]D +9.6° (c 0.24, MeOH); UV (MeOH) λmax (log ε) 195 nm (5.86); IR (KBr) νmax: 3462, 2950, 1584, 1484, 1072 cm⁻¹; ¹H NMR and ¹³C NMR, see Table 1; HR-ESI-MS: m/z 423.1417 [M + H]+, Calcd. 423.1420); CD (c 0.24, MeOH); λ max (5): 450-457.

References


