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Clerodens E–J, antibacterial caffeic acid derivatives from the aerial part of *Clerodendranthus spicatus*

Qing Li [a,1], Ying-Na He [a,1], Xiao-Wei Shi [b], Li-Ying Kang [a], Li-Ying Niu [a], Xin-Guo Wang [a,*], Wei Feng [a,*]

[a] Hebei TCM Formula Granule Engineering & Technology Research Center, Hebei University of Chinese Medicine, Shijiazhuang 050091, China
[b] School of Pharmaceutical Sciences, Hebei Medical University, Shijiazhuang 050017, China

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ABSTRACT

Six new caffeic acid derivatives, Clerodens E–J (**1**–**6**) were isolated from the aerial part of *Clerodendranthus spicatus*. Their structures were elucidated by extensive spectroscopic analysis, including NMR, MS, and ECD data. Compound **1** showed moderate antibacterial activities against drug-resistant strains of bacteria *in vitro*.

1. Introduction

Currently, the overuse of antibiotics is a particular concern in China [1], especially for urinary tract infections frequently caused by drug-resistant strains of bacteria [2]. The pace of new antibiotic development has not kept up with the emergence of antibiotic-resistant bacterial strains, which makes us to pay close attention to herbal antibiotics as an alternative to increasingly ineffective drugs [3]. *Clerodendranthus spicatus* (Thunb.) C.Y. Wu (syn. *Orthosiphonstamineus* Benth.) (Lamiaceae), known as "Kumis kucing" in Southeast Asia, is a popular herbal medicine extensively used for the treatment of nephritis and cystitis [4–5]. The leaves and stems of this plant cultivated in Southern China are used by the name of Yanuomiao as a diuretic tea in Chinese folk medicine to treat urinary infection and lithiasis [6–10]. TGF-β1 antagonistic activity is found to partly responsible for the therapeutic efficacy of this plant to treat renal disease [11]. Previous phytochemical investigation on this species revealed the presence of triterpenes [11], diterpenes [12–18], flavonoids [19], and phenolic acids [20–21]. In the course of our study on bioactive constituents of this plant, antibacterial potential of total phenolic acids of *C. spicatus* (TPC) was evaluated *in vitro*. Further investigation resulted in the isolation of six new caffeic acid derivatives, clerodens E–J (**1**–**6**) (Fig. 1) with an unusual bicyclo [2.2.2] octene moiety. Among these isolates, compound **1** showed moderate antibacterial activities against clinically isolated drug-resistant bacterial strains. Herein, we report the isolation and structure elucidation of the new isolates as well as antibacterial activity evaluation of some new compounds.

2. Experimental

2.1. General experimental procedures

Optical rotations were obtained on a Perkin-Elmer 341 digital polarimeter. UV and IR spectra were recorded on Shimadzu UV2550 and FTIR-8400S spectrometers, respectively. ECD spectra were obtained using a JASCO J-815 spectropolarimeter. NMR spectra were obtained with a Bruker AV III 600 NMR spectrometer (chemical shift values are presented as δ values with TMS as the internal standard). HRESIMS spectra were performed on a LTQ–Obitrap XL spectrometer. Preparative HPLC was performed on a Lumtech K1001 analytic LC equipped with two pumps of K-501, a UV detector of K-2600, and an YMC Pack C_{18} column (250 mm × 10 mm, i.d., 5 μm, YMC Co. Ltd., Japan) eluted with $CH_3OH - H_2O$ at a flow rate of 2 mL/min. C18 reversed-phase silica gel (40-63 μm, Merk, Darmstadt, Germany), Toyopearl HW-40C (50–100 μm, Tosoh Corp., Tokyo, Japan), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), MCI gel (CHP 20P, 75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) and silica gel (100–200 and 300–400 mesh, Qingdao Marine Chemical plant, Qingdao, People's Republic of China) were used for column chromatography. And pre-coated silica gel GF_{254} plates (Zhi Fu Huang Wu Pilot Plant of Silica Gel Development,

* Corresponding authors.
E-mail addresses: wangxinguozy@126.com (X.-G. Wang), rainbow10571@126.com (W. Feng).
[1] These authors contributed equally to this work.

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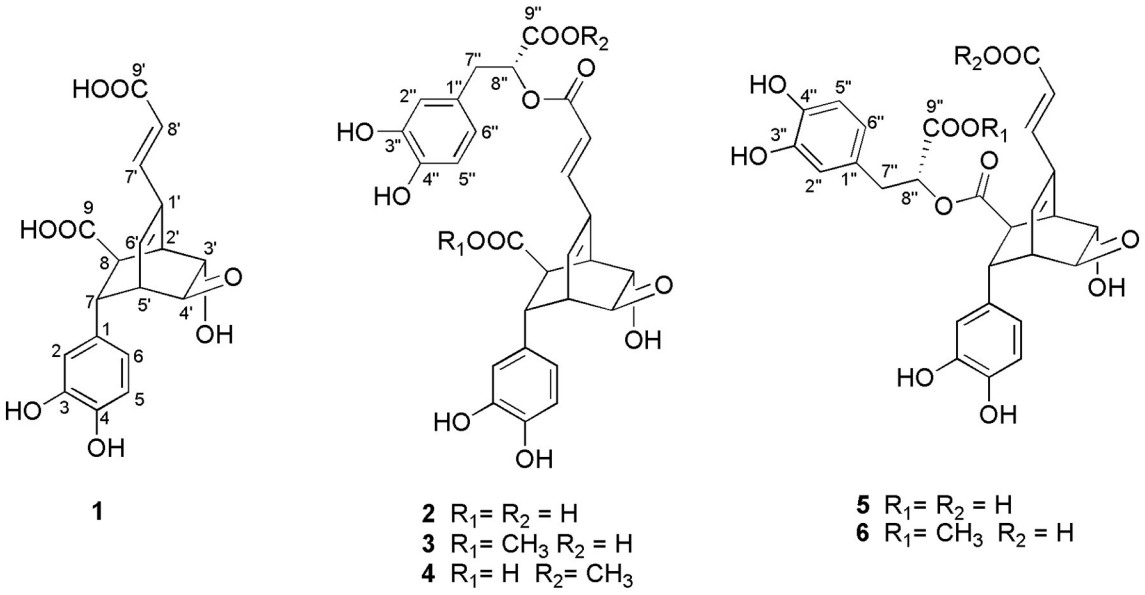

Fig. 1. Structures of compounds 1–6.

Yantai, People's Republic of China) were used for TLC. All solvents used were of analytical grade (Beijing Chemical Works).

2.2. Plant material

The whole plants of *C. spicatus* were collected in Jinghong, Yunnan Province, People's Republic of China, in September 2012, and were authenticated by Prof. Jing-Quan Yuan. A voucher specimen (CS-21209) has been deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences.

2.3. Extraction and isolation

The ground powder of air-dried whole plants of *C. spicatu* (15 kg) was decocted with boiled water (50 L × 2 h × 3) and the solution was then precipitated in 70% ethanol and filtered to yield the supernatant. The supernatant was concentrated under reduced pressure to yield an extract. The extract was subjected to D101 macroporous resin column chromatography using ethanol–water gradient elution, and the eluted fraction with 30–95% ethanol–water elution combined after concentration yielded the total phenolic acids of *C. spicatu* (TPC, 260 g). TPC fraction was further separated by MCI-gel column chromatography with methanol–water gradient elution, giving seven fractions (A–G). Fraction B (20.8 g) was fractionated on HW-40C column chromatography using 40% methanol, giving ten subfractions (*B*1–B10). Subfraction B5 (409.3 mg) were chromatographed by semi-preparative HPLC using MeOH-H_2O (36: 64, *v*/v) to yield compound **1** (20.2 mg, t_R = 25.4 min). Subfraction B8 (532.3 mg) were purified by semipreparative HPLC using MeOH-H_2O (31: 69) to yield **2** (9.1 mg, t_R = 23.2 min). Fraction C (18.8 g) was chromatographed on HW-40C column chromatography using 90% methanol, yielding nine subfractions (C1–C9). Subfraction C3 (507.7 mg) were chromatographed by semipreparative HPLC using MeOH-H_2O (28:72, v/v) to yield **3** (5.7 mg, t_R = 23.4 min) and **4** (4.9 mg, t_R = 28.7 min). Compounds **5** (5.4 mg, t_R = 24 min) and **6** (4.9 mg, t_R = 27 min) were obtained from subfraction C5 (548.2 mg) by semi-preparative HPLC (MeOH − H_2O, 34:66).

2.3.1. Cleroden E (**1**)

Brown amorphous powder; [α]25 D + 378 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 269 (4.22) nm; IR (KBr) ν_{max} 3374, 1720, 1693, 1626, 1523, 1287, 1196 cm^{-1}; for ^1H and ^{13}CNMR spectroscopic data,

Table 1
^1H NMR spectroscopic data of compounds **1**−**6**[a].

Position	1	2	3	4	5	6
2	6.76, s	6.74, s	6.73, s	6.76, s	6.75, d (1.8)	6.73, d (1.8)
5	6.67, d (7.8)	6.67, d (7.8)	6.67, d (8.4)	6.67, d (7.8)	6.67, d (7.8)	6.68, d (7.8)
6	6.63, br d (7.8)	6.63, br d (7.8)	6.60, br d (8.4)	6.64, br d (7.8)	6.54, d (7.8)	6.58, brd (7.8)
7	3.49, s	3.49, s	3.45, dd (7.2, 1.8)	3.49, s	3.23, d (6)	3.31, br s
8	3.51, s	3.49, s	3.53, d (7.2)	3.49, s	3.58,d (7.8)	3.58, d (5.4)
2′	3.81, s	3.79, br s	3.76, br s	3.80, br s	3.77, br s	3.77, br s
3′	3.72, s	3.70, br s	3.71, d (3.0)	3.71, br s	3.66, d (3)	3.70, br s
5′	3.16, d (6.0)	3.15, d (6.6)	3.15, dd (7.2 1.8)	3.17, d (6.0)	3.09, dd (6.6, 2.4)	3.13, d (6.0)
6′	6.69, d (6.0)	6.72, d (6.6)	6.72, d (7.2)	6.70, d (6.0)	6.53, d (6.6)	6.65, br s
7′	7.35, d (15.6)	7.35, d (15.6)	7.31, d (15.6)	7.38, d (15.6)	7.18, d (13.8)	7.26, d (16.2)
8′	6.14, d (15.6)	6.13, d (15.6)	6.12, d (15.6)	6.18, d (15.6)	6.21,d (13.8)	6.16, d (16.2)
2″		6.75, br s	6.73, br s	6.70, br s	6.63, br s	6.59, br s
5″		6.67, d (7.8)	6.67, d (7.8)	6.68, d (7.8)	6.59, d (7.8)	6.62, d (7.8)
6″		6.61, br d (7.8)	6.61, br d (7.8)	6.56, br d (7.8)	6.40, d (7.8)	6.40, d (7.8)
7″		2.96, dd (14.4, 9)	2.96, br s	3.00, br d (14.4)	3.00, d (13.8)	3.05, dd (14.4, 9.0)
		3.09, d (14.4)	3.13, br s	3.03, br d (14.4)	2.79, dd (13.8, 10.0)	3.03, dd (14.4, 4.8)
8″		5.13, br s	5.16, br s	5.19, br s	5.06, br s	5.11, dd (9.0, 4.8)
OCH_3			3.57, s	3.69, s		3.64, s

[a] Data (δ) were measured at 600 MHz in methanol-d_4, *J* in Hz.