Chemical Compounds from the Twigs and Leaves of *Caesalpinia cucullata* Roxb

Xuesong Liang, Dewen Bi, Fengqiu Li and Liqin Wang*

Faculty of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming 650050, China

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**Abstract:** A new stilbene dimer, caesalstilbene A (1), along with twelve known compounds were isolated from the twigs and leaves of *Caesalpinia cucullata* Roxb by means of various chromatographic techniques. Their structures were identified on the basis of NMR spectral analysis and comparing their spectral data with those reported in the literatures. The absolute configuration of 1 was assigned by the comparison of the experimental and calculated electronic circular dichroism spectra. Compound 1 was evaluated for their cytotoxicity on HL-60, SMMC-7721, A-549, MCF-7 and SW-480 human cancer cell lines, but it was inactive. This is the first report of chemical investigation on *Caesalpinia cucullata*.

**Keywords:** *Caesalpinia cucullata*; Leguminaceae; stilbene dimer; caesalstilbene A. © 2019 ACG Publications. All rights reserved.

1. Introduction

*Caesalpinia cucullata* Roxb, belonging to the family Leguminosae, is distributed in south of Yunnan province of China, India, Nepal, Sikkim [1]. There are 17 *Caesalpinia* species widespread in China, and 14 species have long been used in Chinese traditional medicine to reduce swelling and alleviate pain, to treat rheumatism and inflammatory [2]. Because of their characteristic cassane diterpenoids and homoisoflavonoids, whose structures and bioactivities are diverse [2,3,4], the plants of *Caesalpinia* have drawn wide attention. As our continuous investigation on *Caesalpinia* plants [5,6], one new stilbene dimer, caesalstilbene A (1), along with twelve known compounds were isolated from the twigs and leaves of *C. cucullata*. All these compounds were isolated from *C. cucullata* for the first time. Here, we described the isolation and structural elucidation of these compounds.

2. Materials and Methods

2.1. Instrumentation and Reagents

NMR spectra were acquired on Bruker DRX-500 spectrometer. MS data were obtained using a Bruker microTOF spectrometers. Fractions were monitored by TLC on silica gel plates (GF254, Qingdao Puke separation material Co., Ltd., Qingdao, China). Column chromatography (CC) was performed on silica gel (100-200 mesh or 200-300 mesh; Qingdao Puke separation material Co., Ltd., Qingdao, China), Sephadex LH-20 (GE Healthcare) and MCI gel (75–150 mm, Mitsubishi Chemical Corporation, Tokyo, Japan). All solvents used in column chromatography were of industrial grade and used after distillation under vacuum.

* Corresponding author: E-mail: lqwang@ynnu.edu.cn; Phone/Fax: 86-871-65941088
2.2. Plant Material

The twigs and leaves of *Caesalpinia cucullata* were collected from Xishuangbanna, Yunnan, China in November 2015, and identified by Chunfen Xiao (Xishuangbanna Botanical Garden, Chinese Academy of Sciences) to be the same as the voucher specimen (HITBC 017509) deposited at Xishuangbanna Botanical Garden, Chinese Academy of Sciences.

2.3. Extraction

The powdered twigs and leaves (11.5 kg) of *C. cucullata* were extracted with EtOH at room temperature, which afforded a dark residue after evaporation under reduced pressure. The residue was dissolved in H2O and extracted by ethyl acetate (EtOAc). The EtOAc extract (360 g) was subjected to CC (SiO2, 100-200 mesh; petroleum ether/ EtOAc 10:1; 8:1; 6:1; 4:1; 2:1; 1:1; 0:1), to gain ten fractions (Fr. T1-T10), and then it was eluted with CH3OH to afford fraction T1.

Fr. T1 (6.9 g) was subjected to CC (SiO2, petroleum ether/EtOAc 5:1:1) to gain five subfractions (T3a-T3e). T3b (642.9 mg) was subjected to CC (SiO2, petroleum ether/ EtOAc 5:1; CHCl3/CH3COCH3 20:1; Sephadex LH-20, CHCl3/MeOH 1:1) to provide compound 8 (4.2 mg); T3d (538.7 mg) was subjected to CC (SiO2, CHCl3/CH3COCH3 40:1; Sephadex LH-20, CHCl3/MeOH 1:1) to provide compound 3 (45.8 mg).

Fr. T3 (3.6 g) was subjected to CC (SiO2, petroleum ether/EtOAc 5:1) to gain three subfractions (T4a-T4c). T4c (529 mg) was subjected to CC (SiO2, CHCl3/CH3COCH3 20:1, petroleum ether/ EtOAc 5:1) to provide compound 4 (21 mg).

Fr. T4 (12.0 g) was subjected to CC (SiO2, petroleum ether/CH3COCH3 2:1:1) to obtain four subfractions (T5a-T5d). T5a (2.9 g) was subjected to CC (SiO2, petroleum ether/CH3COCH3 2:1; CHCl3/MeOH 15:1; Sephadex LH-20, CHCl3/MeOH 1:1) to provide compound 9 (2 mg). T5d (18.6 mg) was subjected to CC (SiO2, CHCl3/CH3COCH3 8:1; Sephadex LH-20, MeOH) to get compound 6 (4.1 mg).

Fr. T5 (9.3 g) was subjected to CC (SiO2, petroleum ether/CH3COCH3 1:5:1) to gain three subfractions (T6a-T6c). T6b (210 mg) was subjected to CC (Sephadex LH-20, CHCl3/MeOH 1:1; SiO2, CHCl3/MeOH 15:1) to provide compound 7 (6.5 mg).

Fr. T6 (13.4 g) was subjected to CC (SiO2, petroleum ether/CH3COCH3 3:2) to obtain four subfractions (T7a-T7d). T7c (3.5 g) was subjected to CC (SiO2, CHCl3/MeOH 20:1; CHCl3/CH3COCH3 8:1; 6:1) to give compound 12 (20 mg). T7d (3.2 g) was subjected to CC (SiO2, CHCl3/MeOH 20:1; 8:1; Sephadex LH-20, MeOH; SiO2, petroleum ether/EtOAc 2:1) to get compounds 11 (16.3 mg), 2 (120 mg) and 5 (9.7 mg).

Fr. T7 (10.9 g) was subjected to CC (SiO2, petroleum ether/CH3COCH3 3:2) to obtain five subfractions (T8a-T8e). T8c (1.9 g) was subjected to CC (SiO2, CHCl3/MeOH 10:1; Sephadex LH-20, CHCl3/MeOH 1:1) to get compounds 10 (9 mg) and 13 (7 mg).

Fr. T8 (13.8 g) was subjected to CC (SiO2, petroleum ether/CH3COCH3 1:1) to obtain four subfractions (T10a-T10d). T10c (2.3 g) was subjected to CC (SiO2, petroleum ether/CH3COCH3 1:1; CHCl3/MeOH 6:1; 4:1) to get compound 1 (14 mg).

*Caesalpinia* A (I): colorless amorphous solid, UV(MeOH): λmax (log ε) = 202 (2.96), 282 (2.02) nm; δ1H 20.1 -25.89 (c 0.130, CH3OH); Positive ESIMS: m/z 471 [M+H]+, Positive HRESI-MS: 471.1446 [M+H]+ (calc for C28H32O7, 471.1438). 1H-NMR and 13C-NMR data, see Table 1.

3. Results and Discussion

Compound 1 was isolated as brown amorphous solid, with the molecular formula C28H32O7 (18 degrees of unsaturation), as determined by HRESIMS at m/z 471.1446 [M+H]+. The 1H NMR and 13C NMR spectra showed the presence of one set of ortho-coupled aromatic protons derived from a 4-hydroxyphenyl group, one set of aromatic protons coupled in an AX2 system due to a 3,5-dihydroxyphenyl group, one set of aromatic protons coupled in an AX system due to a 2,3,5-substituted phenyl group, two singlet aromatic protons attributed to a 2,4,5-substituted phenyl group, and four aliphatic methines (H-7a, H-8a, H-7b and H-8b). This evidence, together with the limitation...
imposed by eighteen unsaturations, indicated the existence of two ring systems and four benzene rings in 1. These observations suggested that compound 1 was a stilbene dimer with a planar structure similar to that of cararosinol C [7], except that the location of the hydroxyl groups on ring B1 and B2 were not as same as that of cararosinol C. Ring B1 and ring B2 were 3,5-dihydroxy and 2,4,5-substituted phenyl groups respectively, which was verified by the HMBC correlations of H-2b(6b)/C-7b, H-11b/C-7a, H-2a(6a)/C-7a, H-14a/C-8a, H-14b/C-8b, H-7b/C-11a (Figure 1). In its ROESY spectrum, the correlations between H-7b/H-8a, H-7b/H-14b, H-7a/H-8b, and H-7a/H-11b indicated that the hydroxyphenyl groups at C-7a and C-7b were trans-oriented to each other, while they were cis-oriented towards H-8a and H-8b respectively. Therefore, the relative stereostructure of compound 1 was established as shown in Figure 1 (1a or 1b).

Figure 1. The structures of compounds 1a and 1b, the key HMBC and ROESY correlations of 1a

![Figure 1](image)

The absolute configuration of 1 was determined by comparison of the experimentally obtained-, and simulated, electronic circular dichroism (ECD) spectra. The ECD spectra of 1a better matched the experimental ECD spectra of 1 (Figure 2), suggest the (7aR,8aR,8bR,8aS) absolute configuration for 1. Hence, the structure of 1 was elucidated to be 1a, and named as caesalstilbene A.

Compound 2-13 were identified to be trans-resveratrol (2) [8], 2',4'-dihydroxy-4-methoxycal-
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cone (3) [9], isoliquiritinigenin (4) [10], butein (5) [11], 3,2',4'-tri hydroxy-4-methoxychalcone (6) [12], 2',4',4'-tri hydroxy-3'-meth oxychalcone (kukulkanin B) (7) [13], 7-hydroxy-4'-methoxyflavonone (8) [10], naringenin (9) [14], butin (10) [15], aromadendrin (11) [16], apigenin (12) [8], and luteolin (13) [8] (Figure 3), by their 1D-NMR analysis and comparing their spectral data with those reported in the literatures.

The cassane diterpenoids and homoisoflavonoids are the characteristic components of *Caesalpinia* plants [17]. However, our present study showed that the cassane diterpenoids were not found in *Mezoneuron* sub-genus [5,6]. This is the major difference between *Caesalpinia* sub-genus and *Mezoneuron* sub-genus. Thus the cassane diterpenoids might be a unique chemotaxonomic marker for *Caesalpinia* sub-genus. Depth studies need to be carried out.

Using the MTS method reported in the literature [5], compound 1 was tested for their cytotoxicity against the HL-60 (acute leukemia), SMMC-7721 (liver cancer), A-549 (lung cancer), MCF-7 (mammary cancer) and SW-480 (colon cancer) human tumor cell lines, but no activity was noted with IC\textsubscript{50} values more than 40 \(\mu\)M.

Table 1. \(^1\)H NMR (ppm, \(J\) in Hz in parentheses, recorded at 500 MHz) and \(^{13}\)C NMR spectroscopic data (recorded at 125 MHz) of compound 1 (in CD\textsubscript{3}COCD\textsubscript{3})

| Position | \(\delta\)H | \(\delta\)C | Position | \(\delta\)H | \(\delta\)C |
|----------|-----------|-----------|----------|-----------|-----------|
| 1a       | 138.8     | 1b        | 10a      | 122.7     | 10b       |
| 2a/6a    | 6.96 (d, 8.5) | 128.9     | 2b/6b    | 6.15 (d, 2.2) | 106.6     |
| 3a/5a    | 6.73 (d, 8.5) | 116.0     | 3b/5b    | 6.0 (d, 5.5) | 60.4      |
| 4a       | 156.4     | 4b        | 7a       | 4.34 (s)  | 57.8      |
| 7a       | 3.85 (d, 5.5) | 60.0      | 8b       | 3.82 (d, 5.5) | 60.4      |
| 9a       | 150.7     | 9b        | 10a      | 122.7     | 10b       |
| 10a      | 156.5     | 11b       | 11a      | 159.4     | 13b       |
| 12a      | 6.17 (d, 1.7) | 102.4     | 12b      | 6.47 (s)  | 112.3     |
| 13a      | 159.4     | 14b       | 14a      | 6.54 (d, 1.7) | 103.2     |
| 14a      | 103.2     | 14b       | 14a      | 7.00 (s)  | 111.3     |

Figure 3. The structures of compounds 2-13
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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID

Xuesong Liang: 0000-0001-8036-7037
Dewen Bi: 0000-0003-4224-8947
Fengqiu Li: 0000-0002-0676-4226
Liqin Wang: 0000-0002-2440-0186

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