ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. X:X (2019) XX-XX

records of natural products

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

(Received December 05, 2018; Revised April 07, 2019; Accepted Month April 08, 2019)

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.

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 (GF_{254}, 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

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products © Month-Month 2019 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.124.18.12.1088

2.2. Plant Material

The twigs and leaves of *Caesalpinia cucullata* wer2e 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 H_2O and extracted by ethyl acetate (EtOAc). The EtOAc extract (360 g) was subjected to CC (SiO_2, 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.* T_1-T_{10}), and then it was eluted with CH_3OH to afford fraction T_{11}.

Fr.T_3 (6.9 g) was subjected to CC (SiO_2, petroleum ether/EtOAc 5:1-1:1) to gain five subfractions (T_{3a}-T_{3e}). T_{3b} (642.9 mg) was subjected to CC (SiO_2, petroleum ether/ EtOAc 5:1, $CHCl_3$/CH_3COCH_3 20:1; Sephadex *LH-20*, $CHCl_3$/MeOH 1:1) to provide compound **8** (4.2 mg); T_{3d} (538.7 mg) was subjected to CC (SiO_2, $CHCl_3$/CH_3COCH_3 40:1; Sephadex *LH-20*, $CHCl_3$/MeOH 1:1) to provide compound **3** (45.8 mg).

Fr.T_4 (3.6 g) was subjected to CC (SiO_2, petroleum ether/EtOAc 5:1) to gain three subfractions (T_{4a}-T_{4c}). T_{4c} (529 mg) was subjected to CC (SiO_2, $CHCl_3$/CH_3COCH_3 20:1, petroleum ether/ EtOAc 5:1) to provide compound **4** (21 mg).

Fr.T_6 (12.0 g) was subjected to CC (SiO_2, petroleum ether/CH_3COCH_3 2:1-1:1) to obtain four subfractions (T_{6a}-T_{6d}). T_{6a} (2.9 g) was subjected to CC (SiO_2, petroleum ether/CH_3COCH_3 2:1; $CHCl_3$/ MeOH 15:1; Sephadex *LH-20*, $CHCl_3$/MeOH 1:1) to provide compound **9** (2 mg). T_{6d} (18.6 mg) was subjected to CC (SiO_2, $CHCl_3$/CH_3COCH_3 8:1; Sephadex *LH-20*, MeOH) to get compound **6** (4.1 mg).

Fr.T_7 (9.3 g) was subjected to CC (SiO_2, petroleum ether/CH_3COCH_3 1.5:1) to gain three subfractions (T_{7a}-T_{7c}). T_{7b} (210 mg) was subjected to CC (Sephadex *LH-20*, $CHCl_3$/MeOH 1:1; SiO_2, $CHCl_3$/MeOH 15:1) to provide compound **7** (6.5 mg).

Fr.T_8 (13.4 g) was subjected to CC (SiO_2, petroleum ether/CH_3COCH_3 3:2) to obtain four subfractions (T_{8a}-T_{8d}). T_{8c} (3.5 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 20:1; $CHCl_3$/ CH_3COCH_3 8:1-6:1) to give compound **12** (20 mg). T_{8d} (3.2 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 20:1-8:1; Sephadex *LH-20*, MeOH; SiO_2, petroleum ether/EtOAc 2:1) to get compounds **11** (16.3 mg), **2** (120 mg) and **5** (9.7 mg).

Fr.T_9 (10.9 g) was subjected to CC (SiO_2, petroleum ether/CH_3COCH_3 3:2) to obtain five subfractions (T_{9a}-T_{9e}). T_{9d} (1.9 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 10:1; Sephadex *LH-20*, $CHCl_3$/MeOH 1:1) to get compounds **10** (9 mg) and **13** (7 mg).

Fr.T_{10} (13.8 g) was subjected to CC (SiO_2, petroleum ether/ CH_3COCH_3 1:1) to obtain four subfractions (T_{10a}-T_{10d}). T_{10c} (2.3 g) was subjected to CC (SiO_2, petroleum ether/CH_3COCH_3 1:1; $CHCl_3$/MeOH 6:1-4:1) to get compound **1** (14 mg).

Caesalstilbene A (**1**): colorless amorphous solid, UV(MeOH): λ_{max} (log ε) = 202 (2.96), 282 (2.02) nm; $[\alpha]_D^{20.1}$ -25.89 (*c* 0.130, CH_3OH); Positive ESIMS: *m/z* 471 $[M+H]^+$, Positive HRESI-MS: 471.1446 $[M+H]^+$ (calcd for $C_{28}H_{23}O_7$, 471.1438). ^{1}H-NMR and ^{13}C-NMR data, see Table 1.

3. Results and Discussion

Compound **1** was isolated as brown amorphous solid, with the molecular formula $C_{28}H_{22}O_7$ (18 degrees of unsaturation), as determined by HRESIMS at *m/z* 471.1446 [*M* +H] $^+$. The ^{1}H NMR and ^{13}C 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 AX_2 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**).

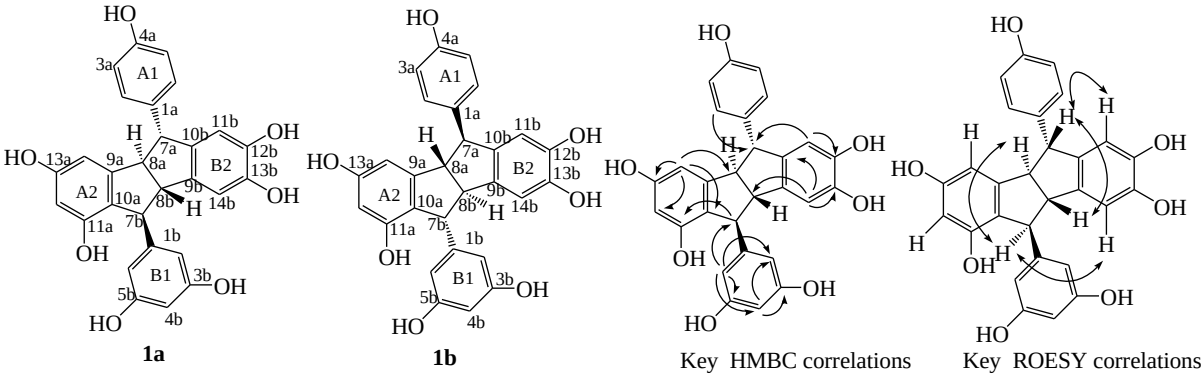

Figure 1. The structures of compounds **1a** and **1b**, the key HMBC and ROESY correlations of **1a**

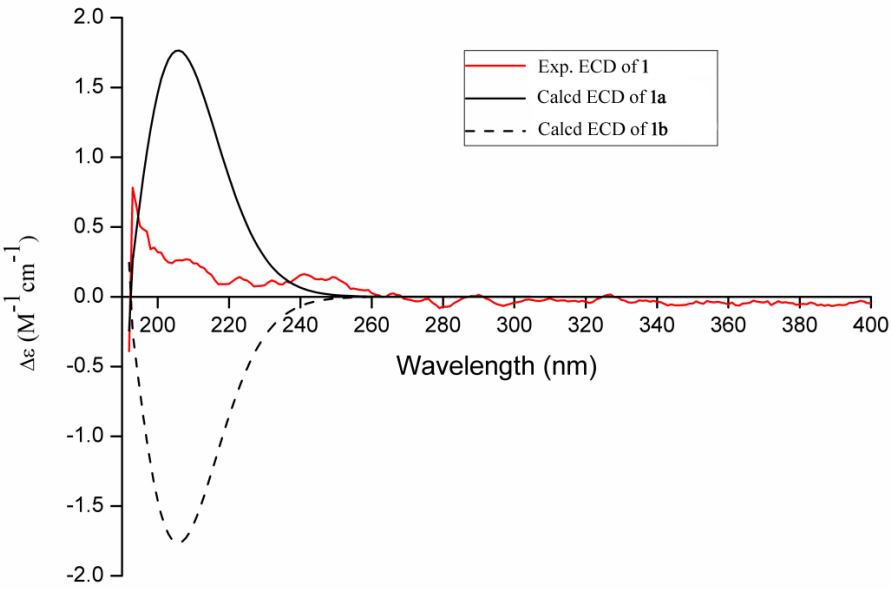

Figure 2. Comparison of experimental ECD spectra of **1** (red solid line) in MeOH and the calculated ECD spectra of **1a** (black solid line) and **1b** (black dotted line) at the B3LYP/6-311+G(d, p) level

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 (7a*R*,8a*R*,8b*R*,8a*S*) 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-methoxyychal-cone (**3**) [9], isoliquiritigenin (**4**) [10], butein (**5**) [11], 3,2',4'-trihydroxy-4-methoxychalcone (**6**) [12],

2',4',4-trihydroxy-3'-methoxychalcone (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_{50} values more than 40 μM.

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_3COCD_3)

Position	δ_H	δ_C	Position	δ_H	δ_C
1a		138.8	1b		138.1
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		159.3
4a		156.4	4b	6.13 (t, 2.2)	101.0
7a	4.34 (s)	57.8	7b	4.40 (s)	55.2
8a	3.85 (d, 5.5)	60.0	8b	3.82 (d, 5.5)	60.4
9a		150.7	9b		150.7
10a		122.7	10b		137.6
11a		156.5	11b	6.47 (s)	112.3
12a	6.17 (d, 1.7)	102.4	12b		146.6
13a		159.4	13b		146.7
14a	6.54 (d, 1.7)	103.2	14b	7.00 (s)	111.3

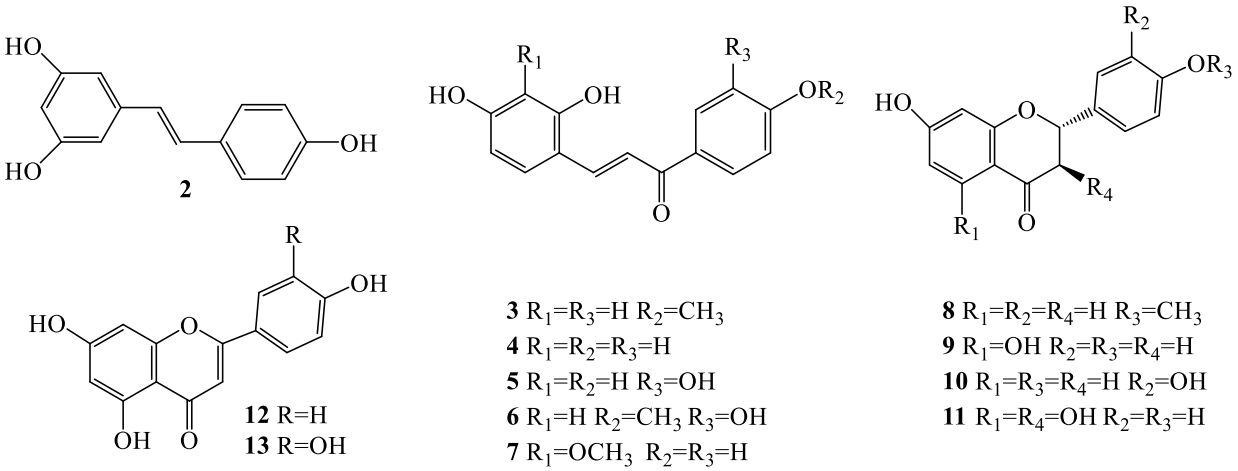

Figure 3. The structures of compounds **2-13**

Acknowledgments

This research was funded by the National Natural Science Foundation of China (No. 31460085).

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

References

[1] Delectis Flora Reipublicae Popularis Sinicae Edita (1988). *Flora Reipublicae Popularis Sinicae*, Beijing: Science Press **39**, 100.
[2] M. Wu, Y. F. Wang, M. L. Zhang, C. H. Huo, M. Dong and W. S. Qin (2011). Chemical constituents of plants from the genus *Caesalpinia*, *Chem. Biodivers*. **8**, 1370-1399.
[3] R. Maurya, M. Ravi, S. Singh and P. P. Yadav (2012). A review on cassane and norcassane diterpenes and their pharmacological studies, *Fitoterapia* **83**, 272-280.
[4] B. Jian, J. Huang, M. J. Guo, P. Zou and X. Q. Tian (2007). Recent advances in the study of natural homoisoflavonoids, *Acta. Pharm. Sin*. **42**, 118-126.
[5] L. J. Zhang, D. W. Bi, J. M. Hu, W. H. Mu, Y. P. Li, G. H. Xia, L. Yang, X. N. Li, X. S. Liang and L. Q. Wang (2017). Four hybrid flavan-chalcones, caesalpinnone A possessing a 10,11-dioxatricyclic [5.3.3.01,6] tridecane bridged system, and caesalpinflavans A-C, from *Caesalpinia enneaphylla*, *Org. Lett.* **19**, 4315-4318.
[6] L. J. Zhang, D. W. Bi, J. H. Wang, G. H. Xia and L. Q. Wang(2019). Chemical constituents from the twigs and leaves of *Caesalpinia enneaphylla*, *J. Chin. Pharm. Sci.* **27**, 864-870.
[7] G. X. Yang, J. T. Zhou and Y. Z. Li (2005). Anti-HIV bioactive stilbene dimers of *Caragana rosea*, *Planta Med*. **71**, 569-571.
[8] J. P. Yi, W. X. Zhu, H. L. Ma and Y. F. Wang (2009). Studies on the chemical constituents from the seeds of *Paeonia suffruticosa* Andr, *Nat. Prod. Res. Dev.* **21**, 604-607.
[9] G. X. Meng, Q. H. Wang and Y. H. Guo (2014). Flavonoid components of *Oxytropis myriophylla* (Pall.) DC, *Nat. Prod. Res. Dev.* **26**, 1614-1617.
[10] Y. He, J. Lu and R. C. Lin (2003). Studies on chemical constituents in root of *Isatis indigotica*, *Chin. Tradit. Herb. Drugs.* **34**, 777-778.
[11] X. Ji, S. L. Wu, T. Z. Jia, Z. H. Guo and H. Y. Gao (2014). Study on chemical constituents of *Myristicae semen*, *Chin. Tradit. Herb. Drugs.* **45**, 3367-3372.
[12] C. F. Wang, J. P. Li, R. R. Li, Y. Zhao, Y. B. Zhang and Z. Z. Zhang (2009). Structural elucidation of three Flavonoids extracted from the rhizomes of *Ligularia vellerea* by NMR spectroscopy, *Chin. J. Magn. Reson.* **26**, 264-271.
[13] X. A. Dominguez, S. Garcia, H. J. Williams, C. Ortiz, A. I. Scott and J.H. Reibenspies (1989). Kuku-lkanins A and B, new chalcones from *Mimosa tenuefolia*, *J. Nat. Prod*. **52**, 864-867.
[14] D. W. Bi, X. S. Liang, G. H. Xia, L. J. Zhang and L. Q. Wang (2018). Flavonoids from the seeds of *Caesalpinia mimosoides*, *J. Chin. Med. Mater*. **41**, 353-356.
[15] M. C. Li, S. A. Tang and H. Q. Duan (2010). Chemical consitituents from *Onychium japonicum*, *Chin. Tradit. Herb. Drugs.* **41**, 685-688.
[16] Z. L. Li, X. H. Lv, X. J. Wang, X. J. Zhou, J. Li and S. H. Qian (2018). Chemical constituents from twig and leaves of Taxus media, *Chin. Tradit. Herb. Drugs*. **49**, 3226-3231.
[17] W. H. Jing, X. X. Zhang, H. X. Zhou, Y. Wang, M. Q. Yang, L. P. Long and H. Y. Gao (2019), Naturally occurring cassane diterpenoids (CAs) of *Caesalpinia*: A systematic review of its biosynthesis, chemistry and pharmacology, *Fitoterapia* **134**, 226-249.