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(+)- and (−)-Cajanusine, a Pair of New Enantiomeric Stilbene Dimers with a New Skeleton from the Leaves of *Cajanus cajan*

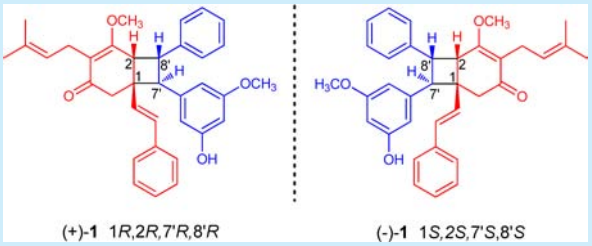
Xiao-Long Li,[†,‡,§] Bing-Xin Zhao,[†,‡,§] Xiao-Jun Huang,[†,‡] Dong-Mei Zhang,[†] Ren-Wang Jiang,[†] Ying-Jie Li,[†] Yu-Qing Jian,[†,‡] Ying Wang,*[,†,‡] Yao-Lan Li,*[,†] and Wen-Cai Ye*[,†,‡]

[†]Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, People's Republic of China

[‡]JNU-HKUST Joint Laboratory for Neuroscience & Innovative Drug Research, Jinan University, Guangzhou 510632, People's Republic of China

Supporting Information

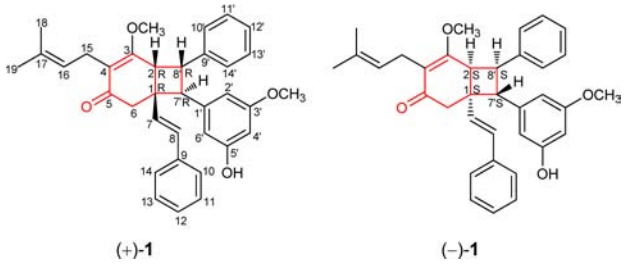
ABSTRACT: A pair of new enantiomeric stilbene dimers, (+)- and (−)-cajanusine [(+)-**1** and (−)-**1**], with a unique coupling pattern were isolated from the leaves of *Cajanus cajan*. Their structures including absolute configurations were elucidated on the basis of comprehensive spectroscopic and single-crystal X-ray diffraction analyses, as well as CD calculations. The plausible biogenetic pathway of **1** was also proposed. Additionally, (±)-**1**, (+)-**1**, and (−)-**1** exhibited inhibitory activities on the growth of human hepatocellular carcinoma cells.



The plant *Cajanus cajan* (Linn.) Millsp. (Leguminosae) is widely distributed and cultivated in southern China. The leaves of this plant have been used as folk medicine for the treatment of diabetes,[1,2] plasmodiosis,[3] sickle cell anemia,[4] hepatic disorders,[5] and avascular necrosis of the femoral head.[6] Previous phytochemical studies on this plant had resulted in the isolation of a number of stilbenes,[7] some of which showed estrogenic,[8] hypocholesterolemic,[9] and antioxidative activities,[10] as well as protective effect on cognitive deficit.[11]

In our continuing search for structurally unique and biologically interesting constituents from the medicinal plants growing in southern China,[12−16] a pair of new enantiomeric stilbene dimers, (+)- and (−)-cajanusine [(+)-**1** and (−)-**1**], along with their biosynthetic precursors [longistyline A (**2**)[17] and 3-methoxy-5-hydroxystilbene (**3**)[18]], were isolated from the leaves of the title plant. Cajanusine (**1**), the skeleton presumably arises from two heterogeneous monomeric stilbenes (**2** and **3**) through a radical addition to form a rare bicyclo[4.2.0]oct-4-en-3-one unit, represents the first example of oligomeric stilbene with a unique coupling pattern between monomeric stilbenes.[18] Herein, we report the isolation and structural elucidation of the enantiomeric stilbene dimers. In addition, the plausible biogenetic pathway of **1** and the cytotoxic effects of (±)-**1**, (+)-**1**, (−)-**1**, **2**, and **3** are also described.

Cajanusine (**1**) was obtained as yellow bulk crystals, $[\alpha]_D^{25}$ ±0° (*c* 0.30, MeOH). The molecular formula of **1** was established as $C_{35}H_{36}O_4$ by its HR-ESI-MS (*m*/*z* 521.2754 [M + H]$^+$, calcd for $C_{35}H_{37}O_4$ 521.2751). The UV spectrum of **1** showed absorption maxima at λ_{max} 206 and 258 nm. The IR spectrum suggested the presence of a hydroxyl group (3432 cm^{-1}) and aromatic ring (1596 and 1445 cm^{-1}). The analysis of



NMR spectra revealed that **1** possessed 35 carbons, including two monosubstituted benzene rings [δ_H 7.28 (10H, overlapped); δ_C 137.5, 137.0, 128.8, 128.7, 128.6, 128.0, 127.7, and 126.4], a trisubstituted benzene ring [δ_H 6.27 (3H, overlapped); δ_C 160.9, 157.4, 139.8, 107.7, 105.8, and 100.0], a *trans*-1,2-disubstituted vinyl unit [δ_H 6.50 (1H, d, *J* = 16.2 Hz) and 6.25 (1H, d, *J* = 16.2 Hz); δ_C 132.1 and 129.2], three methines [δ_H 4.21 (1H, dd, *J* = 11.7 Hz, 9.3 Hz), 4.01 (1H, d, *J* = 11.7 Hz), and 3.85 (1H, d, *J* = 9.3 Hz); δ_C 50.9, 44.9, and 42.4], two methoxyls [δ_H 3.64 and 3.25 (each 3H, s); δ_C 55.3 and 55.1], two vinyl methyls [δ_H 1.73 and 1.70 (each 3H, s); δ_C 26.0 and 17.9], and a carbonyl (δ_C 197.3). All the above spectral data suggested that **1** was a dimeric stilbene with an additional C_5 unit. With the aid of 1D and 2D NMR experiments, all the ^{1}H and ^{13}C NMR signals of **1** were assigned as shown in Table 1.

The ^{1}H−^{1}H COSY data of **1** revealed the presence of three spin-coupling systems shown in bold in Figure 1. In the HMBC spectrum, correlations between H-2 and C-4/C-6/C-7,

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Table 1. NMR data of 1 (in $CDCl_3$, J in Hz)[a]

no.	δ_H	δ_C	no.	δ_H	δ_C
1		42.2	1′		139.8
2	3.85 (d, 9.3)	42.4	2′	6.22	105.8
3		169.2	3′		160.9
4		120.6	4′	6.27	100.0
5		197.3	5′		157.4
6	a 2.84 (d, 17.1)	43.9	6′	6.32	107.7
	b 2.55 (d, 17.1)		7′	4.01 (d, 11.7)	50.9
7	6.25 (d, 16.2)	132.1	8′	4.21 (dd, 11.7, 9.3)	44.9
8	6.50 (d, 16.2)	129.2	9′		137.5
9		137.0	10′	7.28	128.6
10	7.28	126.4	11′	7.28	128.7
11	7.28	128.8	12′	7.28	128.0
12	7.28	127.7	13′	7.28	128.7
13	7.28	128.8	14′	7.28	128.6
14	7.28	126.4	3-OMe	3.25 (s)	55.1
15	a 3.06 (dd, 13.8, 7.2)	22.2	3′-OMe	3.64 (s)	55.3
	b 3.16 (dd, 13.8, 7.2)				
16	5.22 (dd, 7.2, 7.2)	122.3			
17		131.7			
18	1.70 (s)	17.9			
19	1.73 (s)	26.0			

[a]Overlapped signals were reported without designating multiplicity.

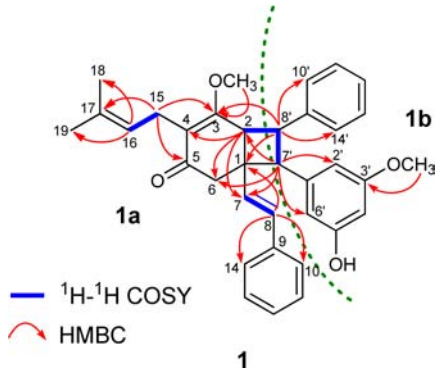

Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of **1**.

between H-8 and C-1/C-10/C-14, between H-15 and C-3/C-5/C-17, between H-16 and C-18/C-19, as well as between 3-OCH_3 (δ_H 3.25) and C-3 led to the establishment of the substructure **1a** (Figure 1). Furthermore, the HMBC correlations between H-7′ and C-2′/C-6′, between H-8′ and C-10′/C-14′, as well as between 3′-OCH_3 (δ_H 3.64) and C-3′ verified the structure of fragment **1b** (Figure 1). In addition, the HMBC correlations between H-7′ and C-2/C-6/C-7 as well as between H-8′ and C-1/C-3 indicated that the two fragments **1a** and **1b** were connected via C-2−C-8′ and C-1−C-7′ bonds to form a cyclobutane unit (Figure 1). The ROESY spectrum of **1** displayed significant NOE correlations between H-2 and H-8′/H-8 as well as between H-8′ and H-8/H-2′/H-6′, suggesting that these protons had the same orientation.

The structure and relative configuration of **1** were confirmed by a single-crystal X-ray diffraction experiment[19] (Figure 2). However, the crystal structure of **1** was found to exhibit a centrosymmetric space group $P\bar{1}$, which suggested the presence of a racemic mixture. Subsequently, **1** was resolved into two enantiomers, (+)-**1** and (−)-**1**, in a ratio of 1:1 by a chiral

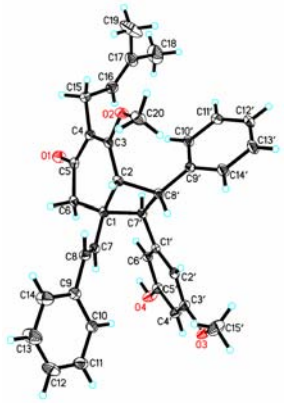

Figure 2. X-ray structure of **1**.

HPLC column (see Supporting Information). The CD spectra of (+)-**1** and (−)-**1** displayed similar signal intensity but opposite Cotton effects, confirming their enantiomeric relationship (Figure 3).

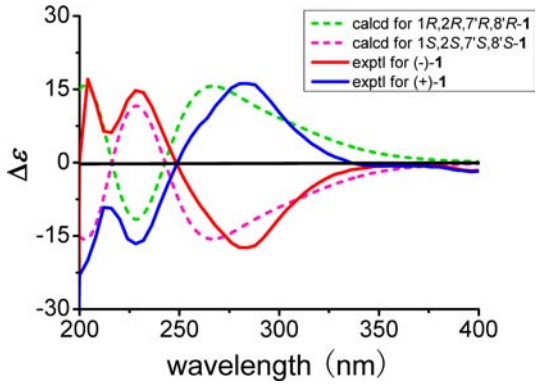

Figure 3. Experimental and calculated CD spectra of (+)-**1** and (−)-**1**.

The absolute configurations of the two enantiomers of **1** were determined by the CD spectra coupled with the quantum chemical CD calculation in Gaussion 09 software.[20] The relative structures obtained from X-ray diffraction experiment were used as the stable conformers for CD calculation, which were performed by the TDDFT [B3LYP/6-311++G(2d,p)] method. The predicted CD spectra of 1*R*,2*R*,7′*R*,8′*R*-**1** and 1*S*,2*S*,7′*S*,8′*S*-**1** revealed good agreement with the experimental ones of (+)-**1** and (−)-**1** (Figure 3). Therefore, the stereostructures of (+)-**1** and (−)-**1** were, respectively, established as 1*R*,2*R*,7′*R*,8′*R* and 1*S*,2*S*,7′*S*,8′*S*.

The plausible biogenetic pathway of **1** could be proposed as shown in Scheme 1.[21] First, the precursor **2** was deprotonated to generate a free radical **A**. The unpaired electron of radical **A** could be dispersed to positions *ortho* and *para*, successively, to form two resonance-stabilized free radicals **B** and **C**. Then, radical **C** attached **3** from above or below of the molecular plane through a intermolecule radical addition reaction to form the C-2−C-8′ bond and generate two radicals **D** and **E** with two new chiral centers (C-2 and C-8′). Subsequently, radicals **F** and **G** were yielded from radicals **D** and **E**, respectively, through an intramolecular radical cyclization reaction to form a rare bicyclo[4.2.0]oct-4-en-3-one ring system. Finally, radicals **F** and **G** were terminated reductively to give products (+)-**1** and (−)-**1**, respectively. It is noteworthy that **1** is the first example

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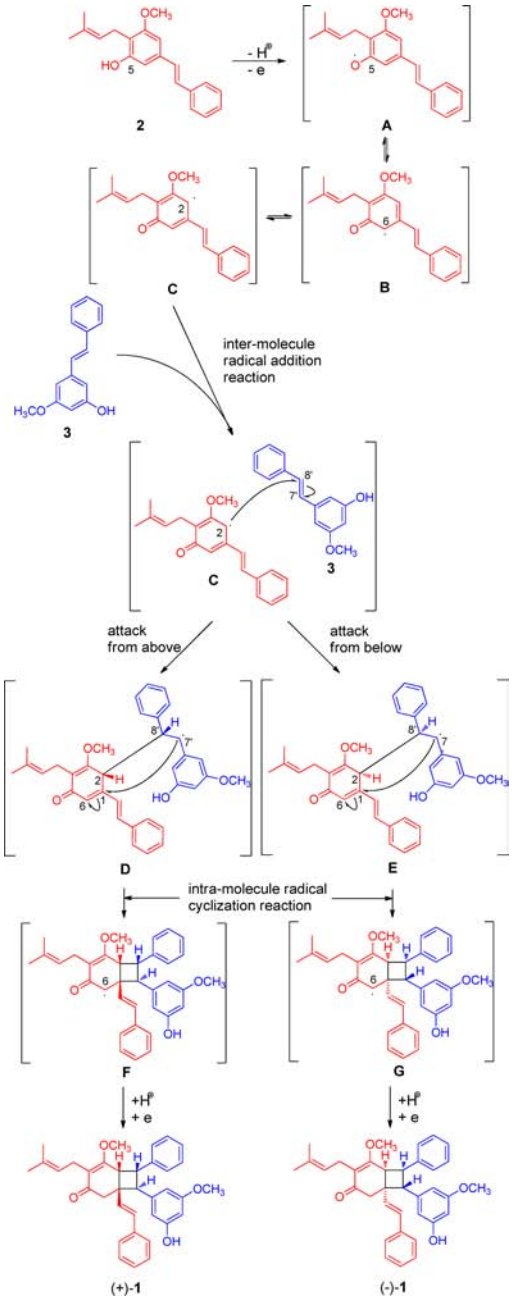
Scheme 1. Plausible Biogenetic Route of (+)-1 and (−)-1



of oligomeric stilbene with a unique coupling pattern between monomeric stilbene units.[18]

The MTT colorimetric assay was performed to detect the antitumor activities of **1**−**3** in doxorubicin-sensitive and -resistant human hepatocellular carcinoma cells (HepG2 and HepG2/ADM) as previous described.[12] As shown in Table 2, stilbene dimers (±)-**1**, (+)-**1**, and (−)-**1** exhibited significant cytotoxic effects on both HepG2 and HepG2/ADM cells, indicating that difference in stereostructure of **1** might have no effect on its cytotoxic activities. Stilbene monomers **2** and **3** showed weak antiproliferative activities on both HepG2 and HepG2/ADM cells. These data indicated that stilbene dimers might exert more potent activity than stilbene monomers. Interestingly, the results (Table 2) showed that **2** could selectively inhibit the growth of HepG2 cells but not drug-resistant HepG2/ADM cells, whereas **3** was more sensitive to

Table 2. Cytotoxicity Values of (±)-1, (+)-1, (−)-1, 2, and 3 in Human Hepatocellular Carcinoma Cells

	IC_{50} ± SD (μM)	
compounds	HepG2	HepG2/ADM
(±)-**1**	16.23 ± 6.12	20.45 ± 4.31
(+)-**1**	17.46 ± 5.03	27.24 ± 7.88
(−)-**1**	18.03 ± 3.08	13.29 ± 3.59
2	35.84 ± 0.08	>50
3	>50	34.61 ± 3.77
doxorubicin[a]	0.41 ± 0.01	63.90 ± 4.34

[a]Positive control.

HepG2/ADM cells. Inspired by these findings, we presumed that the involvement of isopentenyl in a stilbene monomer might induce changes in molecular mechanism for its cytotoxic activity.

■ ASSOCIATED CONTENT

Supporting Information

Detailed description of the experimental procedure, a listing of UV, IR, HR-ESI-MS and NMR spectra, CIF files, CD data, and bioassay data of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: wangying_cpu@163.com.
*E-mail: tliyl@jnu.edu.cn.
*E-mail: chyewc@gmail.com.

Author Contributions

§These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

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