Two New Stilbenoids from *Cajanus cajan*

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Two new stilbenoids cajanotone and cajanamide A (**1**–**2**), together with another six known ones (**3**–**8**) and four known dihydroflavones (**9**–**12**), have been isolated from the leaves of *Cajanus cajan*. Their structures were elucidated based on spectroscopic studies. A possible pathway to the new compounds **1** and **2** has been proposed. *In vitro* cytotoxicities of selected compounds against cancer cell lines HepG2, MCF-7 and A549 have been evaluated. Compounds **7** and **8** show strong cytotoxity against all the tested cell lines (with IC_{50} values in the range of 3.5 – 6.0 μM), and compounds **1** and **3** showed strong to moderate activity against the three cell lines.

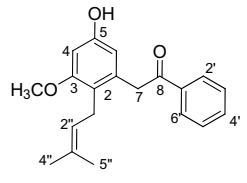
Key words: Cajanus cajan, Pigeon Pea, Stilbenoid, Dihydroflavone, Cytotoxicity

Introduction

Stilbenoids are bibenzyl compounds produced by the mixed phenylpropanoid/polyketide biosynthetic pathway. Increasing attention has been drawn to them (especially resveratrol) due to their various biological activities, such as antioxidant, anticancer, estrogenic, and antibacterial activity [1]. Pigeon pea [*Cajanus cajan* (L.) Millsp.], which belongs to the *Cajanus* genus, one of the most valuable perennial or annual leguminous food crops in Asia, Africa and some parts of tropical and subtropical areas of the world, has been reported to contain stilbenoids, flavonoids, coumarin, and other kinds of constituents [2 – 7], yet the number of reported compounds is quite small. Our interest in the bioactivities of stilbenoids and the attempt to enrich the chemical constituents in pigeon pea led to the isolation of two new stilbenoids, cajanotone (**1**) and cajanamide A (**2**), six known ones (**3**–**8**) and four known dihydroflavones (**9**–**12**) (Fig. 1). In this paper, we describe the isolation and structure elucidation of the new compounds and the cytotoxicity of compounds **1**, **3** and **6**–**8**.

Results and Discussion

Compound **1** was obtained as a pale-yellow oil. Its molecular formula was determined as $C_{20}H_{22}O_3$ by HREIMS ($m/z = 310.1561$; calcd. 310.1563 for $C_{20}H_{22}O_3$, $[M]^+$). The ^1H and ^{13}C NMR spectra (Table 1) of the compound showed the presence of two benzene rings [$\delta_H = 6.19$ (1H, d, $J = 2.2$ Hz), 6.34 (1H, d, $J = 2.2$ Hz), 7.96 (2H, d, $J = 7.5$ Hz), 7.56 (1H, t, $J = 7.5$ Hz), 7.44 ppm (2H, t, $J = 7.5$ Hz)], an isoprenyl moiety [$\delta_H = 3.24$ (2 H, d, $J = 6.5$ Hz), 5.00 (1H, t, $J = 6.5$ Hz), 1.59 ppm (6H, s)], a methoxy [$\delta_H = 3.76$ ppm (3H, s)] and a carbonyl group ($\delta_C = 198.1$ ppm), which were very similar to those of longistylin C (compound **8**) [8], except for the absence of two *trans* olefinic protons and the appearance of a carbonyl carbon resonance at $\delta_C = 198.1$ ppm and aliphatic signals at $\delta_H = 4.21$ ppm (2H, s) and $\delta_C = 42.8$ ppm, suggesting that the carbonyl and the aliphatic carbons are linked to the two benzene rings. This assumption was further evidenced by HMBC spectra (Fig. 2), displaying the following correlations: H-2′ and H-6′ ($\delta_H = 7.96$ ppm) with C-8, H-7 ($\delta_H = 4.21$ ppm) with C-2 ($\delta_C = 121.0$ ppm) and C-6



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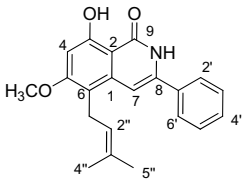

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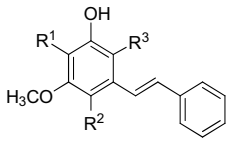
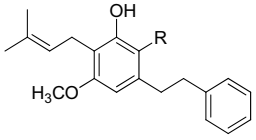
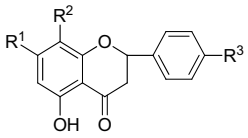


3 R^1 = prenyl, R^2 = H, R^3 = COOH
6 R^1 = R^2 = R^3 = H
7 R^1 = prenyl, R^2 = R^3 = H
8 R^2 = prenyl, R^1 = R^3 = H



4 R = COOH
5 R = H



9 R^1 = OCH_3, R^2 = R^3 = H
10 R^1 = R^3 = OCH_3, R^2 = H
11 R^1 = OCH_3, R^2 = H, R^3 = OH
12 R^1 = OH, R^2 = prenyl, R^3 = H

Fig. 1. Structures of compounds **1**–**12**.

	1			**2**	
Position	δ_C	δ_H	Position	δ_C	δ_H
1	135.1 (s)		1	137.1 (s)	
2	121.0 (s)		2	104.3 (s)	
3	158.7 (s)		3	161.6 (s)	
4	98.1 (d)	6.34 (d, 2.2)	4	96.8 (d)	6.57 (s)
5	154.5 (s)		5	161.4 (s)	
6	109.0 (d)	6.19 (d, 2.2)	6	113.1 (s)	
7	42.8 (t)	4.21 (s)	7	102.0 (d)	6.78 (s)
8	198.1 (s)		8	139.6 (s)	
			9	167.1 (s)	
1′	136.7 (s)		1′	133.8 (s)	
2′,6′	128.4 (d)	7.96 (d, 7.5)	2′,6′	126.8 (d)	7.75 (m)
3′,5′	128.6 (d)	7.44 (t, 7.5)	3′,5′	128.8 (d)	7.48 – 7.54 (m)
4′	133.2 (d)	7.56 (t, 7.5)	4′	129.4 (d)	7.48 – 7.54 (m)
1″	24.8 (t)	3.24 (d, 6.5)	1″	23.1 (t)	3.46 (d, 6.4)
2″	123.2 (d)	5.00 (t, 6.5)	2″	123.2 (d)	5.03 (t, 6.4)
3″	131.2 (s)		3″	130.4 (s)	
4″	17.8 (q)	1.59 (s)	4″	17.8 (q)	1.78 (s)
5″	25.6 (q)	1.59 (s)	5″	25.4 (q)	1.61 (s)
3-OMe	55.6 (q)	3.76 (s)	3-OMe	56.0 (q)	3.87 (s)
			10-NH		11.76 (s)
			5-OH		13.37 (s)

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of compound **1** (in $CDCl_3$) and **2** (in [D_6]DMSO). Chemical shifts δ in ppm, multiplicities and J values (Hz) in parentheses.

($\delta_C = 109.0$ ppm). These data also indicated that the carbonyl carbon was adjacent to the mono-substituted benzene ring, thus, the structure was deduced and named as cajanotone.

Compound **2** was obtained as colorless needles in MeOH, its molecular formula was established by HREIMS ($m/z = 335.1519$; calcd. 335.1516 for $C_{21}H_{21}O_3N$, $[M]^+$). From the ^{1}H and ^{13}C NMR spectra, the signals at $\delta_H = 13.37$ (s), $\delta_H = 11.76$ (s) and $\delta_C = 167.1$ ppm suggested the presence of a chelated hydroxyl group and an amide function; five aromatic protons ($\delta_H = 7.75 - 7.48$ ppm) indicated the presense of a mono-substituted benzene ring, and an isoprenyl moiety [$\delta_H = 5.03$ (1H, t, $J = 6.4$), 3.46 (2H, d,

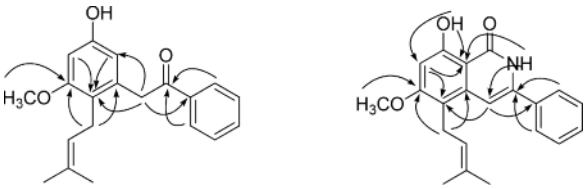


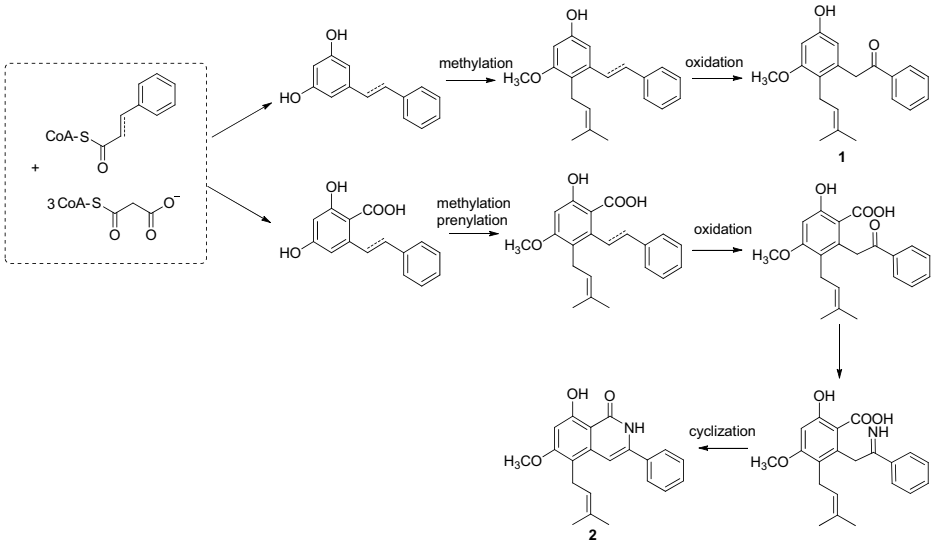
Fig. 2. Key HMBC correlations of compounds **1** and **2**.

$J = 6.4$), 1.78 (3H, s), 1.61 ppm (3H, s)], a carbonyl group ($\delta_C = 167.1$ ppm) and another benzene ring [$\delta_H = 6.57$ ppm (1H, s)] were inferred. The combined analysis of the data (HREIMS, ^{1}H and ^{13}C NMR, HSQC and HMBC) suggested that this compound had a skeleton similar to that of isocoumarin [9], except for the replacement of a lactone unit by a lactam. How the mono-substituted benzene ring was linked to the isocoumarin-like moiety was solved by the HMBC spectrum (Fig. 2), showing correlations between H-2′, H-6′ [$\delta_H = 7.75$ (m)] and C-8 ($\delta_C = 139.6$), H-7 [$\delta_H = 6.78$ (1H, s)] and C-1′ ($\delta_C = 133.8$ ppm). Compound **2** has been identified and named cajanamide A.

By comparison of their spectroscopic data with literature values, the known compounds **5**–**12** were identified as cajaninstilbene acid (**3**) [6, 8], amorfrutin A (**4**) [10], 3-methoxy-2-(3-methylbut-2-enyl)-5-(2-phenylethyl)phenol (**5**) [11], pinosylvin monomethyl ether (**6**) [8], longistylin A (**7**) [8], longistylin C (**8**) [8], pinostrobin (**9**) [4], naringenin 4′,7-dimethyl ether (**10**) [12], naringenin 7-methyl ether (**11**) [13], and 5,7-dihydroxy-8-prenylflavone (**12**) [14].

Stilbenoids are produced by three malonyl-CoAs and one cinnamoyl-CoA/*p*-coumaroyl-CoA *via* stilbene synthase (STS, belonging to the polyketide synthase family). All the stilbenoids isolated here are without substituents on the B-rings (pinosylvin type), suggesting that their precursors might just be cinnamoyl-CoA or/and dihydrocinnamoyl-CoA [15]. The new compounds **1** and **2** were formed by modifications of simple stilbenes, a plausible biogenetic pathway was proposed as shown in Scheme 1 by reference to the literature [16].

The cytotoxicity of compounds **1**, **3** and **6**–**8** against the human hepatoma cell line HepG2, human breast adenocarcinoma MCF-7 and human lung cancer cell line A549 was evaluated by the MTT method [17] with doxorubicin as the positive control. Compounds **7** and **8** exhibited strong cytotoxic activity against all the tested cell lines (with IC_{50} values ranging from 3.5 to 6.0 μM), while compounds **1** and **3** showed strong cytotoxicity against A549 cells (with IC_{50} values of 5.9 and 4.4 μM respectively), but moderate cytotoxicity against HepG2 and MCF-7 cell lines (with IC_{50} values from 12.2 to 17.9 μM); compound **6** showed strong cytotoxicity against MCF-7 (with an IC_{50} value of 8.8 μM) and A549 (with an IC_{50} value of 7.4 μM) cell lines and moderate activity against HepG2 cells, with an IC_{50} value of 15.5 μM (Table 2).



Scheme 1. Possible biogenetic pathway to **1** and **2**.

Table 2. Cytotoxicity data (IC_{50}, μM, mean ± SD) of selected compounds against tumor cell lines[a].

Compounds	HepG2	MCF-7	A549
1	12.2 ± 0.3	17.9 ± 0.4	5.9 ± 0.3
3	12.6 ± 0.3	14.1 ± 0.3	4.4 ± 0.2
6	15.5 ± 0.3	8.8 ± 0.2	7.4 ± 0.2
7	3.5 ± 0.1	3.5 ± 0.1	6.0 ± 0.2
8	4.0 ± 0.2	4.5 ± 0.2	4.4 ± 0.1
Doxorubicin	0.6 ± 0.1	2.0 ± 0.1	1.1 ± 0.1

[a] Results are expressed as IC_{50} values in μM; data were obtained from triplicate experiments, and doxorubicin was used as a positive control.

Experimental Section

General

Optical rotations were recorded on a Perkin-Elmer 341 polarimeter with MeOH as solvent. IR spectra were collected from KBr discs on a WQF-410 FT-IR spectrophotometer. UV spectra were measured in MeOH on a Perkin-Elmer Lambda 650 UV/Vis spectrophotometer. NMR spectra were recorded on a Bruker AM-400 NMR instrument with TMS as internal standard. ESIMS data were taken on a MDS SCIEX API 2000 LC/MS/MS apparatus (Applied Biosystems Inc., Forster, CA/USA). HRMS data were obtained on an MAT95XP mass spectrometer. Silica gel (200 – 300 mesh, Qingdao Marine Chemical Inc., Qingdao/China) and MCI gel CHP 20P (75 – 150 μm, Mitsubishikasei, Tokyo/Japan) were used for column chromatography.

Plant material

The leaves of *Cajanus cajan* (20 kg) were collected in Wenshan, Yunnan, P. R. China in August, 2009. The sample was identified by Prof. Fu-Wu Xing of the South China Botanical Garden. A voucher specimen (SCIB 090912) was deposited at the key laboratory of plant resources conservation and sustainable utilization, South China Botanical Garden, Chinese Academy of Sciences.

Extraction and isolation

The air-dried, milled plant material (20 kg) was extracted by ethanol (3 × 25 L, each 3 d) at room temperature and filtered. The filtrate was evaporated *in vacuo* to afford a residue, which was suspended in H_2O (10 L) and then partitioned with chloroform (3 × 10 L) to afford a chloroform extract (500 g). The chloroform extract was subjected to a silica gel CC (100 – 200 mesh), eluting with petroleum ether-acetone (from 1 : 0 to 0 : 1) to afford fractions Fr 1 – 6. Fr 3 (50 g) was applied to a silica gel CC (200 – 300 mesh) eluted in a step gradient manner with petroleum ether-acetone (from 10 : 0 to 8 : 2) to yield compounds **7** (120.5 mg) and **9** (513.4 mg). Compounds **6** (125.7 mg), **8** (65.5 mg), **10** (15.4 mg), and **12** (20.8 mg) were obtained from Fr 4 (45 g) by repeated silica gel CC eluted with petroleum ether-acetone (from 10 : 0 to 8 : 2). Fr 6 (40 g) was divided into five subfractions by silica gel CC (200 – 300 mesh) using a solvent of petroleum ether-acetone (from 9 : 1 to 6 : 4), and each subfraction was subjected to a MCI-gel CHP 20P column (eluted with methanol), then applied to a silica gel CC (200 – 300 mesh), eluting with petroleum ether-acetone (9 : 1) to give compounds **1** (102.5 mg), **2** (8.9 mg), **3** (55.7 mg), **4** (15.0 mg), **5** (10.4 mg), and **11** (32.8 mg).

*Cajanotone (**1**)*: Pale-yellow oil. – UV/Vis (MeOH): λ_{max} (MeOH) ($\log \varepsilon_{max}$) = 207 (4.19), 283 (2.78) nm. – IR (KBr): ν = 3384, 1673, 1608, 1465, 1326, 1197 cm^{-1}. – ^1H and ^{13}C NMR data: see Table 1. – MS ((+)-ESI): m/z = 333 $[M+Na]^+$. – HRMS (EI, 70 eV): m/z (%) = 310.1561 (100) (calcd. 310.1563 for $C_{20}H_{22}O_3$, $[M]^+$).

*Cajanamide A (**2**)*: Colorless needles (MeOH). – UV/Vis (MeOH): λ_{max} (MeOH) ($\log \varepsilon_{max}$) = 203 (3.77), 208 (3.65), 221 (3.56), 263 (3.52), 344 (3.11) nm. – IR (KBr): ν = 3421, 3170, 1654, 1455, 1309, 1116 cm^{-1}. – ^1H and ^{13}C NMR data: see Table 1. – MS ((+)-ESI): m/z = 336 $[M+H]^+$. – HRMS (EI, 70 eV): m/z (%) = 335.1519 (100) (calcd. 335.1516 for $C_{21}H_{21}O_3N$, $[M]^+$).

Cytotoxicity assay

The cytotoxicity of compounds **1**, **3** and **6–8** was determined by the MTT colorimetric assay as described by Mosmann [17]. Human lung cancer cell line (A549), human breast carcinoma cell line (MCF-7) and human hepatoma cell line (HepG2) were used. Cells were plated at 1×10^4 cells per well in 96 well microtiter plates and incubated for 48 h at 37 °C, 5% CO_2. Each tumor cell line was treated with each test compound at various concentrations in triplicate for incubation for 48 h, doxorubicin (Shanghai Bo'ao Biotech Co., Ltd, Shanghai/China) was used as a positive control. 10 μL MTT reagent (5 mg mL^{-1}) was added, and the incubation was continued at 37 °C for 4 h, then the MTT reagent was removed, and DMSO (150 μL) was added to dissolve the formazan crystals. The absorbance was measured at 570 nm in a microplate reader (Bio-Rad 680). MTT solution in DMSO (without cells and medium) was used as a blank control. The half-maximum inhibitory concentration (IC_{50}) values were calculated by the software SPSS 16.0 from the reduction of absorbance in the control assay. The assay was performed in triplicate, and the data were presented as mean ± S.D in Table 2.

Acknowledgement

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