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New Dibenzocyclooctadiene Lignan from *Kadsura induta* and Their Cytotoxic Activities

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Kadsura induta A. C. Smith (Schisandraceae) is distributed in South China and Vietnam and commonly used for the treatment of rheumatic arthritis, traumatic injury, gastric and duodenal ulcer, dysmenorrhea, and abdominal pain in traditional folk medicines. Previous phytochemical investigations of this plant have yielded lignans and triterpenes.[1,2] Several biological activities such as antiviral and anti-HIV activities have been reported.[2,3] As part of our continuing research into the biologically active constituents of *K. induta* leaves, we report herein the isolation, structural elucidation, and evaluation of cytotoxic activities of one new dibenzocyclooctane lignan and six known compounds from the leaves of *K. induta*.

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{33}H_{36}O_{11}$ by HR-ESI-MS at *m/z* 631.2172 $[M+H]^+$ (Calcd $C_{33}H_{37}O_{11}$ for 631.2150). The ^{1}H-NMR spectrum of **1** showed signals for five aromatic protons at δ_H 7.32 (t, 8.0), 7.46 (d, 8.0), and 7.51 (t, 8.0), assigned to benzoyl moiety, two singlet aromatic protons at δ_H 6.53 (s) and 6.85 (s), two oxygenated methine protons at δ_H 5.77 (s) and 5.88 (s), two oxygenated methylene protons at δ_H 5.63 (d, 1.5) and 5.77 (d, 1.5), four methoxy protons at δ_H 3.32, 3.61, 3.86, and 3.95 as listed in Table 1. The ^{13}C-NMR and DEPT spectra of **1** revealed two carbonyl, twelve quaternary, ten methine, two methylene, four methoxy, and three methyl carbons. The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of schizanrin F (**2**) except for the position of ester group at C-9.[4] The HMBC correlations between H-4 (δ_H 6.85) and C-2 (δ_C 141.1), C-3 (δ_C 151.9), C-5 (δ_C 129.6), C-6 (δ_C 85.2), and C-16 (δ_C 121.8); between H-11 (δ_C 6.53) and C-9 (δ_C 83.4), C-10 (δ_C 132.9), C-12 (δ_C 148.8), C-13 (δ_C 135.5), and C-15 (δ_C 120.2) confirmed the presence of biphenyl rings. In addition, the HMBC correlations from methoxy protons at δ_H 3.61, 3.86, 3.95, and 3.32 to C-1 (δ_C 151.4), C-2 (δ_C 141.1), C-3 (δ_C 151.9), and C-14 (δ_C 140.5), respectively, confirmed that four methoxy groups were at C-1, C-2, C-3, and C-14. The HMBC correlations between methylene protons (δ_C 5.63 and 5.77) and C-12 (δ_C 148.8) and C-13 (δ_C 135.5) suggested the connection of a methylene carbon to C-12 and C-13 via two oxygen atoms. The HMBC correlations between H-6 (δ_H 5.88) and C-7″ (δ_C 164.7); between H-9 (δ_H 5.77) and C-1′ (δ_C 172.3) confirmed the benzoyl and propioyl groups were at C-6 and C-9, respectively. In addition, the COSY correlations, H-9/H-8/8-Me; H-2′/H-3′; and H-2″/H-3″/H-4″ were observed (Figure 2). The above evidence confirmed the constitution of **1**. The CD spectrum of **1** showed the positive Cotton effect at 212 nm and negative Cotton effect at 245 nm, consistent to those of schizanrin F,[4] suggesting the configuration of the biphenyl group to be *S*. The NOESY correlations between H-4 (δ_H 6.85) and H-6 (δ_H 5.88); between H-9 (δ_H 5.77) and H-11 (δ_H 6.53) and H-8 (δ_H 2.32) confirmed the twist-boat-chair conformation for cyclooctadiene ring[5] and the orientations for protons H_α-6, H_β-8, and H_β-9 (Figure 2). The remain configuration of methyl group

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **1** in $CDCl_3$

Pos.	δ_C	δ_H (mult., *J* in Hz)	Pos.	δ_C	δ_H (mult., *J* in Hz)
1	151.4	–	7-Me	28.8	1.40 (s)
2	141.1	–	8-Me	17.1	1.31 (d, 7.0)
3	151.9	–	-OCH_2O-	100.8	5.63 (d, 1.5)
					5.77 (d, 1.5)
4	110.3	6.85 (s)	1-OMe	60.5	3.61 (s)
5	129.6	–	2-OMe	60.4	3.86 (s)
6	85.2	5.88 (s)	3-OMe	56.0	3.95 (s)
7	74.0	–	14-OMe	58.6	3.32 (s)
8	43.2	2.32 (m)	1″	129.3	–
9	83.4	5.77 (s)	2″, 6″	129.4	7.46 (d, 8.0)
10	132.9	–	3″, 5″	127.9	7.32 (t, 8.0)
11	101.7	6.53 (s)	4″	132.8	7.51 (t, 8.0)
12	148.8	–	7″	164.7	–
13	135.5	–	1′	172.3	–
14	140.5	–	2′	26.8	1.77 (dq, 7.5, 17.5)
15	120.2	–			1.90 (dq, 7.5, 17.5)
16	121.8	–	3′	8.4	0.86 (t, 7.5)

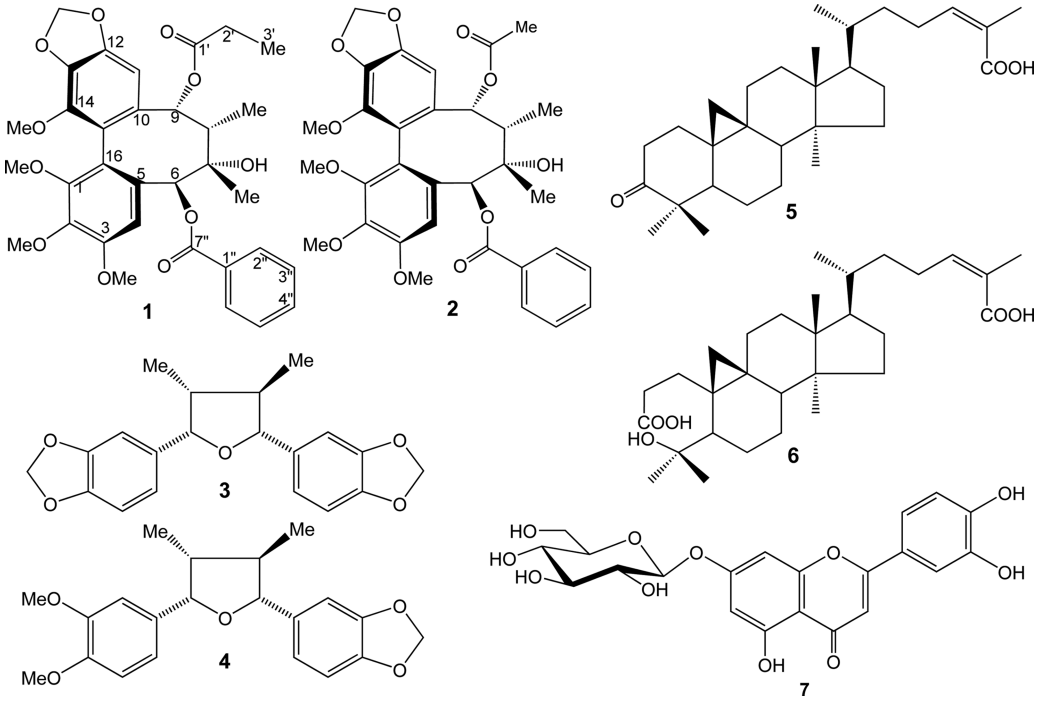

Figure 1. Structures of isolated compounds **1**-**7** from the leaves of *K. induta*.

at C-7 was proved by NOESY correlation between 7-Me (δ_H 1.39) and H-8 (δ_H 2.32). Consequently, the structure of **1** was elucidated and named schizanrin O.

The known compounds were identified as schizanrin F (**2**),[4] rel-(8*R*,8'*R*)-dimethyl-(7*S*,7'*R*)-bis(3,4-methylenedioxyphenyl)tetrahydro-furan (**3**),[6] 3,4-dimethoxy-3′,4′-methylenedioxy-7,7′-epoxilignan (**4**),[7] schizandronic acid (**5**),[8] lancifoic acid A (**6**),[9] and luteolin 7-*O*-β-D-glucopyranoside (**7**)[10] (Figure 1). Their structures were established on the basis of spectral and chemical evidences, which were in agreement with those reported in literature.

Compounds **1**-**7** were evaluated for cytotoxic activities by MTT assay using three human cancer cell lines, OVCAR, HT-29, and A-549. Compounds **3**, **5**, and **6** exhibited moderate cytotoxic effect on the tested cell lines with IC_{50} values ranging from 19.6 to 82.6 μM (Table 2), compared with a mitroxantrone, an anticancer agent used as a positive control exhibiting cytotoxic activities with IC_{50} values of 8.4 ± 0.9 μM (OVCAR), 3.1 ± 0.3 μM (HT-29), and 7.2 ± 0.5 μM (A-549). Compound **4** exhibited moderate activities with IC_{50} values of 32.0 ± 4.1 μM (HT-29) and 66.2 ± 4.5 μM (A-549) without any activity on OVCAR cell line. The remaining compounds did not show cytotoxicity.

Table 2. The effects of compounds **3**-**6** on the growth of human cancer cell lines

Compound	IC_{50} (μM)		
	OVCAR	HT-29	A-549
3	73.6 ± 3.7	48.7 ± 2.3	56.4 ± 3.6
4	> 100	32.0 ± 4.1	66.2 ± 4.5
5	19.6 ± 2.0	33.3 ± 1.4	48.6 ± 2.6
6	34.8 ± 1.5	32.9 ± 0.8	82.6 ± 3.7
Mitoxantrone[a]	8.4 ± 0.9	3.1 ± 0.3	7.2 ± 0.5

[a]Mitoxantrone was used as positive controls. Data presented is the mean ± SD of samples run in triplicate.

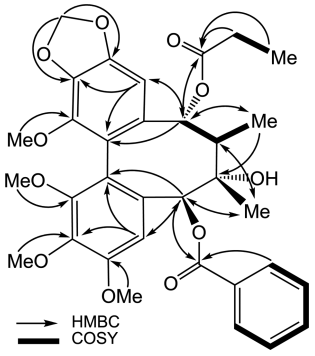

Figure 2. Important HMBC and COSY correlations of compound **1**.

Experimental

General. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. The NMR spectra were recorded using a Bruker DRX 500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz), and the FAB-MS using a JEOL JMS-HX/HX110A tandem mass spectrometer. The HR-ESI-mass spectra were obtained using an AGILENT 6550 iFunnel Q-TOF LC/MS system. Column chromatography was performed using a silica-gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or YMC RP-18 resins (30-50 μm, Fujisilisa Chemical Ltd.), and thin layer chromatography (TLC) using a pre-coated silica-gel 60 F254 (0.25 mm, Merck) and RP-

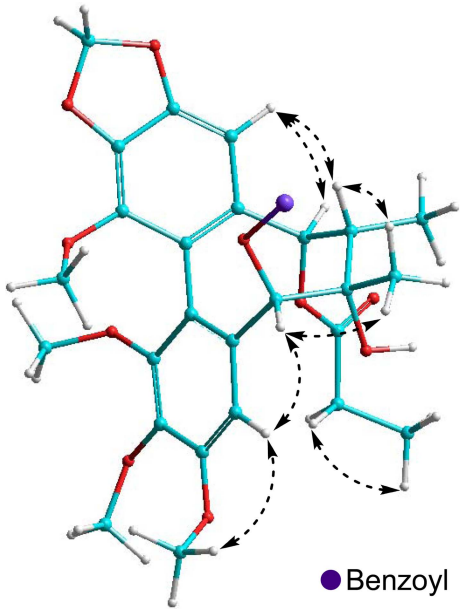

Figure 3. Important NOESY correlations of compound **1**.

18 F_{254S} plates (0.25 mm, Merck).

Plant Material. The leaves of *K. induta* were collected in Tamdao National Botanical Park, Vietnam during July 2012, and identified by Dr Bui Van Thanh, Institute of Ecology and Biological Resources, VAST. A voucher specimen (KI1207) was deposited at the Herbarium of the Institute of Chemistry, VAST.

Extraction and Isolation. The leaves of *K. induta* (1.5 kg) were extracted with MeOH by the sonication for 3 times at room temperature to yield 56.0 g of a dark solid extract, which was then suspended in water and successively partitioned with *n*-hexane and ethyl acetate (EtOAc) to obtain the *n*-hexane (KI1), EtOAc (KI2), and water (KI3) layers. KI1 was chromatographed on a silica gel column and eluted with gradient elution of *n*-hexane–acetone (40:1, 20:1, 10:1, 5:1, and 1:1) to obtain fractions, KI1A–KI1E. KI1B was chromatographed on a silica gel column eluting with *n*-hexane–EtOAc (15:1) to yield **2** (0.5 g). KI2 was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (100:1, 50:1, 25:1, 10:1, and 5:1) to obtain fractions KI2A–KI2E. KH2B was chromatographed on an YMC column eluting with acetone–water (2:1) to yield **1** (8.8 mg). KH2C was chromatographed on an YMC column eluting with MeOH–water (5:1) to yield **3** (8.0 mg) and **4** (6.0 mg). KH2D was chromatographed on an YMC column eluting with acetone–water (2:1) to yield **5** (120.0 mg) and **6** (100.0 mg). KH2E was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (6:1) to yield **7** (117.0 mg).

Schizanrin O (1): A white amorphous powder, $[\alpha]_D^{25}$: −56 (*c* 0.1, $CHCl_3$), CD (*c* 1×10^{-3} M, MeOH) $\Delta\varepsilon_{245} = -3.4$, $\Delta\varepsilon_{212} = +1.8$, HR-ESI-MS found at *m/z* 631.2172 (Calcd $C_{33}H_{37}O_{11}$ for 631.2150), ^{1}H- and ^{13}C-NMR: see Table 1.

Cytotoxicity Assay. The effect of compounds **1-7** on the growth of human cancer cells was determined by measuring the cytotoxic activity using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.[11] The A-549 (human lung cancer), HT-29 (human colon cancer) and OVCAR (human ovarian cancer) cell lines were obtained from the Korea Cell Line Bank (KCLB) and were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 U/mL and 100 g/mL, respectively) at 37 °C in a humidified 5% CO_2 atmosphere. The MTT assays were performed as follows: human cancer cells (1.5-2.5 × 10^5 cells/mL) were treated for 3 days with 1, 10, 50 and 100 μM of the isolated compounds. Mitoxantrone was used to final concentrations of 1, 3, 10 and 20 μM as a reference. After incubation, 0.1 mg (50 μL of a 2 mg/mL solution) MTT (Sigma, Saint Louis, MO, USA) was added to each well and the cells were then incubated at 37 °C for 4 h. The plates were centrifuged at 1000 rpm for 5 min at room temperature and the media was then carefully aspirated. Dimethylsulfoxide (150 μL) was then added to each well to dissolve the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (Amersham Pharmacia Biotech., USA). All the experiments were performed three times and the mean absorbance values were calculated. The results are expressed as the percentage of inhibition that produced a reduction in the absorbance by the treatment of crude extract or solvent fractions compared to the untreated controls. A dose-response curve was generated and the inhibitory concentration of 50% (IC_{50}) was determined for each compound as well as each cell line.

Supporting Information. The NMR, and MS spectra of **1** are available as Supporting information.

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Declaration of Interest. The authors report no conflicts of interest.

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