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NOTE

A New Anthraquinones from *Cassia fistula* and its Cytotoxicity

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A new anthraquinone, cassiquinone A (**1**), was isolated from the stems of *Cassia fistula*. Its structure was elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. Compound **1** was tested for its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) and compound **1** showed high cytotoxicity against A549 and MCF7 cell with IC_{50} values of 8.2 and 6.5 μM, respectively.

Keywords: Anthraquinone, *Cassia fistula*, Cytotoxicity.

Cassia fistula L., (Leguminosae) is an ornamental tree with beautiful yellow flowers[1]. In China, it has been used as traditional Chinese medicine by people of Dai nationality, who lived in Xishuangbanna, Yunnan province for treatment of diarrhea, gastritis, ringworm and fungal skin infections[2,3]. Previous phytochemical studies of *C. fistula* have shown the presence of anthraquinones[4,5], steroids[6], chromones[7,8] and flavonoids[9,10]. Motivated by a search for new bioactive metabolites from local plants, our group investigated the chemical constituents of the stems of *C. fistula* growing in Xishuangbanna Prefecture, which led to the isolation and characterization of a new anthraquinone derivative (**1**). This paper deals with the isolation, structural characterization and the cytotoxicity of these compounds.

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H NMR, ^{13}C NMR and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10-40 μm, Qingdao Marine Chemical Inc., China). Second separation was performed by an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

The stems of *Cassia fistula L.*, (Leguminosae) were collected in Xishuangbanna Prefecture, Yunnan Province, People's Republic of China, in September 2012. The identification of the plant material was verified by Prof. Wu SG (Xishuangbanna Botanical Garden). A voucher specimen (YMU-2012-9-15) has been deposited in our laboratory.

Extraction and isolation. The air-dried and powdered leaves and stems of *C. fistula* (4.2 kg) were extracted four times with 70 % acetone (4 × 5 L) at room temperature and filtered. The crude extract (224 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a MeOH-$CHCl_3$ gradient system (9:1, 8:2, 7:3, 6:4, 5:5), to give five fractions A-E. The further separation of fraction C (7:3, 18.5 g) by silica gel column chromatography, eluted with $(CH_3)_2CO$-$CHCl_3$ (7:3, 6:4, 1:1, 3:7, 2:8), yielded the mixtures C1-C5. The subfraction C3 (1:1, 2.28 g) was subjected to preparative HPLC (30 % MeOH, flow rate 12 mL/min) to give **1** (11.5 mg).

Cassiquinone A (1): $C_{17}H_{18}O_9$, obtained as white powder; $[\alpha]_D^{24.8}$ -148 (c 0.4, MeOH); UV (MeOH) λ_{max} nm (log ε) 358 (1.22), 312 (1.46) and 252 (3.12), 210 (3.74) nm; ν_{max} cm^{-1}: 3428, 3015, 2958, 2876, 1720, 1610, 1538, 1462, 1374, 1145; ^{1}H NMR and ^{13}C NMR (Table-1). HRESIMS *m/z* $[M+Na]^+$ 389.0842 (calcd for $C_{17}H_{18}NaO_9$ for 389.0849).

The air-dried and powdered stems of *C. fistula* (4.2 kg) was extracted with 70 % aqueous acetone (4 × 5.0 L) at room temperature and filtered to yield a filtrate, which was succe-

ssively evaporated under reduced pressure to obtain a crude extract (224 g). This crude extract was subjected repeatedly to column chromatography on Silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compound **1**. Its structure was shown in Fig. 1. The ^{1}H NMR and ^{13}C NMR data of **1** were listed in Table-1.

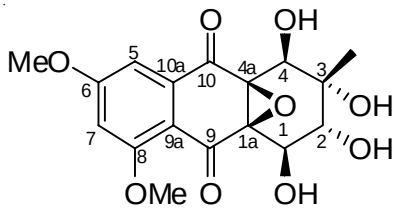

Fig. 1. Structure of compound **1**

TABLE-1
^{1}H NMR AND ^{13}C NMR DATA OF COMPOUND **1**
(DMSO-d_6, 500 AND 125 MHz)

No.	δ_C (m)	δ_H (m, J = Hz)
1	67.8 d	4.46 dd (7.8, 6.0)
2	72.2 d	3.24 dd (7.8, 6.0)
3	68.9 s	
4	66.4 d	4.50 d (7.2)
5	117.1 d	6.86 d (2.4)
6	166.4 s	
7	103.2 d	6.78 d (2.4)
8	163.8 s	
9	192.1 s	6.90, s
10	190.3 s	
1a	68.2 s	
4a	73.1 s	
9a	108.2 s	
10a	132.7 s	
3-Me	22.1 q	1.16 s
6-OMe	56.2 q	3.89 s
8-OMe	56.0 q	3.85 s
1-OH		5.22 d (6.0)
2-OH		4.73 d (6.6)
3-OH		4.58 s
4-OH		5.56, d (7.2)

Cassiquinone A (**1**) was obtained as a white powder, with the molecular formula $C_{17}H_{18}O_9$ (nine degrees of unsaturation) from HRESIMS data combined with ^{1}H NMR and ^{13}C NMR spectroscopic data (Table-1). In the ^{1}H NMR spectrum, two meta-coupled aromatic hydrogens at δ_H 6.86 (d, J = 2.4 Hz) and 6.78 (d, J = 2.4 Hz), three oxygenated methine signals at δ_H 4.50 (d, J = 7.2), 4.46 (dd, J = 7.8, 6.0 Hz) and 3.24 (dd, J = 7.8, 6.6 Hz), two methoxy groups at δ_H 3.89 s and 3.85 s and one singlet methyl group at δ_H 1.12 s were observed. In the ^{13}C NMR spectrum, two carbonyl carbons (δ_C 192.1 s and 190.2 s), six olefinic carbons (δ_C 117.1 d, 166.4 s, 103.2 d, 163.8 s and 192.1 s), six O-bearing carbons (δ_C 67.8 d, 72.2 d, 68.9 s, 66.4 d, 68.2 s and 73.1 s), two methoxy carbons (δ_C 56.2 s and 56.0 s) and one methyl carbon (δ_C 21.6) were observed. These spectroscopic features suggested that compound **1** has a hydroanthraquinone skeleton[11]. The ^{1}H NMR and ^{13}C NMR data (Table-1) were very similar to those of auxarthrol C[11], except for the appearance of a methoxy group (δ_C 56.2 and δ_H 3.85) and the absence of a phenolic hydroxy group in compound **1**. These data indicated that one phenolic hydroxy group in auxarthrol A was replaced by a methoxy group in compound **1**. Two methoxy groups located at C-6 and C-8 were supported by the HMBC correlations (Fig. 2) of the methoxy protons (δ_H 3.89) with C-6 (δ_C 166.4) and (δ_H 3.85) with C-8 (δ_C 163.8), respectively. Thus, the structure of compound **1** was determined.

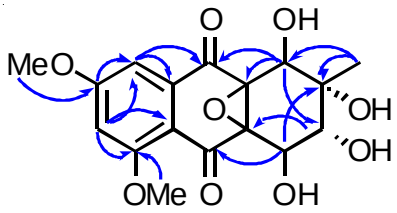

Fig. 2. Key HMBC correlations ( ) of compound **1**

Since some phenolic compounds are known to exhibit potential cytotoxicity[12-14], the cytotoxicity of compounds **1** was tested using a previously reported procedure[15,16]. The cytotoxic abilities against NB4, A549, SHSY5Y, PC3 and MCF7 tumor cell lines by MTT-assay with taxol as the positive control. The results revealed that compound **1** showed cytotoxicity against A549 and MCF7 cell with IC_{50} values of 8.2 and 2.5 μM.

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