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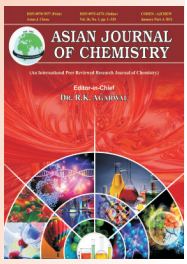

NOTE

A New Anthraquinone from the Fruit of *Cassia fistula* and Its Cytotoxicity

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A new anthraquinone, fistulaquinone A (**1**) was isolated from the fruits of *Cassia fistula*. Its structure was elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. This new compound **1** was tested for its cytotoxicity and it showed potential cytotoxicity against NB4 and PC3 cell with IC_{50} values of 6.3 and 5.8 μM.

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]. Motivated by a search for new bioactive metabolites from local plants, our group investigated the chemical constituents of the fruits of *C. fistula* growing in Xishuangbanna Prefecture, which led to the isolation and characterization of a new anthraquinone (**1**). This paper deals with the isolation, structural characterization and the cytotoxicity of this new compound.

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-, ^{13}C 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 × 250, 7.0 μm) column and DAD detector.

Fruits of *Cassia fistula* L., (Leguminosae) were collected in Xishuangbanna Prefecture, Yunnan Province, People's Republic of China, in September 2011. The identification of the plant material was verified by Prof. Yuan. N (Xishuangbanna Botanical Garden). A voucher specimen (YNNI-2010-9-28) has been deposited in our laboratory.

Extraction and isolation: Air-dried and powdered fruits of *C. fistula* (2.2 kg) were extracted four times with 70 % acetone (4 × 5 L) at room temperature and filtered. The crude extract (126 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 A (9:1, 22.6 g) by silica gel column chromatography, eluted with $(CH_3)_2CO$-$CHCl_3$ (9:1, 8:2, 7:3, 6:4, 1:1), yielded the subfraction A1-A5. The subfraction A1 (9:1, 5.6 g) was subjected to preparative HPLC (65 % MeOH, flow rate 12 mL/min) to give **1** (14 mg).

Fistulaquinone A (1): $C_{20}H_{14}O_7$, Obtained as yellow powder; UV (MeOH), λ_{max} (log ε) 368 (3.57), 282 (4.16), 258 (3.83), 210 (4.36) nm; IR (KBr, ν_{max}, cm^{-1}) 3395, 2926, 2873, 1690, 1652, 1608, 1560, 1487, 1423, 1368, 1273, 1161, 1131, 1068, 876, 763; ^{1}H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table-1; ESI-MS (positive ion mode) *m/z* 389 $[M + H]^+$; HR-ESI-MS (positive ion mode) *m/z* 389.0632 $[M + H]^+$ (calcd 389.0637 for $C_{20}H_{14}NaO_7$).

The air-dried and powdered fruits of *C. fistula* (2.2 kg) was extracted with 70 % aqueous acetone (4 × 5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtain a crude extract (126 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 structures were shown in Fig. 1. The ^{1}H- and ^{13}C NMR data of the compound **1** was listed in Table-1.

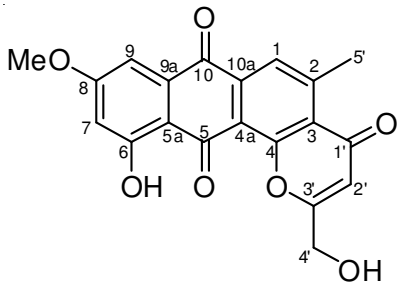

Fig. 1. Structure of compound **1**

TABLE-1
^{1}H AND ^{13}C NMR DATA OF COMPOUND **1** (δ IN ppm, IN $CDCl_3$)

No.	δ_C (m)	δ_H (m, *J* = Hz)	No.	δ_C (m)	δ_H (m, *J* = Hz)
1	124.5 d	7.58, s	9a	121.5 s	–
2	144.4 s	–	10	182.3 s	–
3	133.3 s	–	10a	125.2 s	–
4	155.2 s	–	1'	182.6 s	–
4a	117.6 s	–	2'	108.5 d	6.50, s
5	184.5 s	–	3'	168.6 s	–
5a	113.3 s	–	4'	62.3 t	4.46, s
6	160.5 s	–	5'	18.6 q	2.08, s
7	107.2 d	6.96, s	-OMe-8	55.9 q	3.81, s
8	166.4 s	–	Ar-OH-6	–	12.28, s
9	109.2 d	7.13 s	–	–	–

Compound 1 was isolated as a yellow powder: High-resolution ESIMS analysis gave a quasi-molecular ion at *m/z* 389.0632 $[M + H]^+$, consistent with a molecular formula of $C_{20}H_{14}O_7$, which indicated 14 degrees of unsaturation. The UV spectrum of **1** exhibited absorption bands at 368, 282, 258 and 210 nm, highly suggesting the existence of aromatic chromophore[10]. Strong absorption bands accounting for hydroxy (3395 cm^{-1}), carbonyl (1690, 1652 cm^{-1}) and aromatic groups (1608, 1560 and 1487 cm^{-1}) could also be observed in its IR spectrum. The ^{1}H NMR spectrum of **1** (Table-1) showed the presence of one phenolic hydroxy proton (δ_H 12.28), one methoxy proton (δ_H 3.81), four singlet aromatic protons (δ_H 7.58, 6.96, 7.13 and 6.50) and two aliphatic protons contributed by one methyl singlet (δ_H 2.08), one O-methylene singlet (δ_H 4.46). In the ^{13}C NMR spectrum of **1** (Table-1), 14 sp^2 carbon signals, including three oxygenated quaternary sp^2 carbon signals (δ_C 155.2, 160.5 and 166.4) and two carbonyl carbon signals (δ_C 184.5 and 182.3) were observed, which highly suggested the presence of anthraquinone core[10].

The additional carbons account for the remaining substituents, a methoxy group (δ_C 55.9 s), a hydroxymethyl chromone ring (δ_C 182.6 s, 108.5 d, 168.6 s, 62.3 t)[11], and a methyl carbon (δ_C 18.6) on the anthraquinone ring. The substituents and their location on the anthraquinone ring were established by analysis of the HMBC spectra of 1 (Fig. 2). The HMBC correlations from a methyl singlet (δ_H 2.08) to C-1 (δ_C 124.5), C-2 (δ_C 144.4) and C-3 (δ_C 133.3) established the location of a methyl group at C-2. HMBC correlations between the hydroxy proton (δ_H 12.28) and C-6 (δ_C 160.5), C-7 (δ_C 107.2) and C-5a (δ_C 113.3), led to the assignment of the phenolic hydroxy group at C-6. The methoxy group located at C-8 was supported by the HMBC correlation of the methoxy proton (δ_H 3.81) with C-8 (δ_C 166.4). Additionally, H-2′ (δ_H 6.50) showed correlation with the carbon signal of C-3 (δ_C 133.3) clearly indicated that the hydroxymethyl chromone ring should be located between C-3 and C-4. On the basis of the above evidence, the structure of **1** was established as shown and given the trivail name of fistulaquinone A.

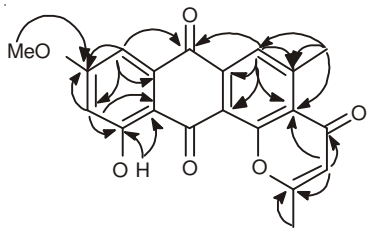

Fig. 2. Key HMBC (→) correlation of **1**

Compound **1** were tested for its cytotoxicity against five tumor cells line (NB4, A549, SHSY5Y, PC3 and MCF7) using a previously reported procedure[12]. The results showed that **1** exhibited potential cytotoxicity against NB_4 and PC_3 cell with IC_{50} values of 6.3 and 5.8 μM.

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