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ANTHRAQUINONES FROM *CASSIA FISTULA* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITY

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Abstract – Two new anthraquinones, 9,11-dihydroxy-2-(hydroxymethyl)-5-methyl-4*H*-naphtho[2,3-*h*]chromene-4,7,12-trione (**1**) and 9,11-dihydroxy-2,5-dimethyl-4*H*-naphtho[2,3-*h*]chromene-4,7,12-trione (**2**), together with five known anthraquinones (**3**-**7**) were isolated from the stems of *Cassia fistula*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. Compounds **1**-**7** were tested for their anti-tobacco mosaic virus activity. The results showed that compound **7** exhibited high anti-TMV activity with inhibition rate of 35.2%. The inhibition rate is higher than that of positive control. The other compounds also showed potential anti-TMV activities with inhibition rates in the range of 15.2–24.8%, respectively.

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] Flavonoids possessing anti-tobacco mosaic virus (anti-TMV) have been isolated from *C. fistula* grown in De Hong Prefecture by our group.[9] 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 two new (**1** and **2**) and five

known (**3**-**7**) anthraquinones derivatives. This paper deals with the isolation, structural characterization, and the anti-tobacco mosaic virus (anti-TMV) activities of these compounds.

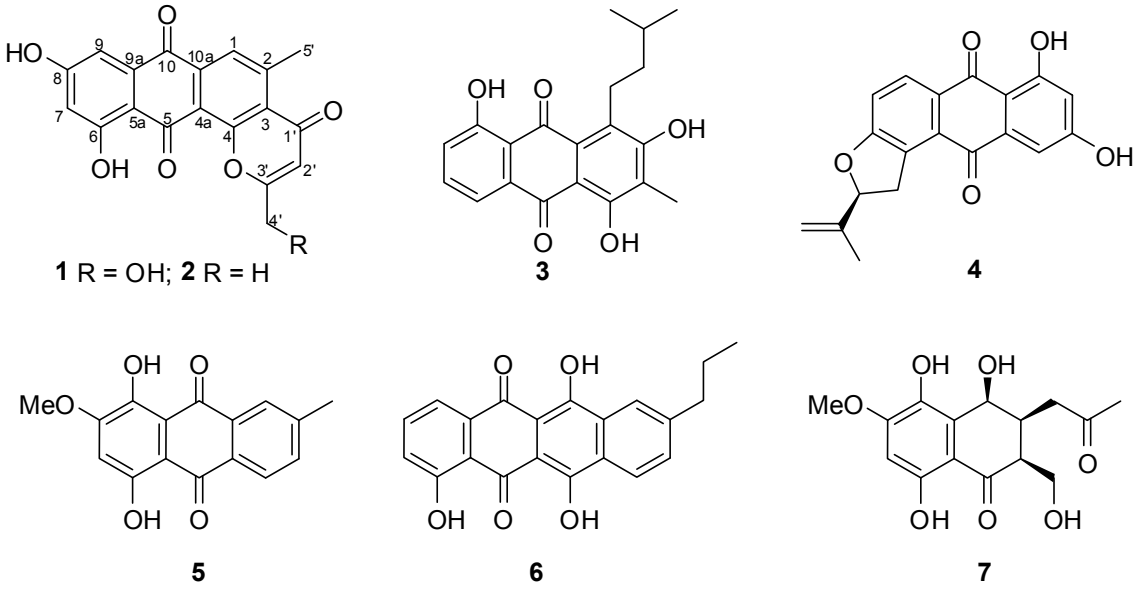

Figure 1. The structures of anthraquinones from *C. fistula*

The air-dried and powdered stems of *C. fistula* (4.8 kg) was extracted with 70% aqueous acetone (4 × 5.0 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure to obtain a crude extract (258 g). This crude extract was subjected repeatedly to column chromatography on Silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1**-**7**. Their structures were shown in **Figure 1**. The ^{1}H- and ^{13}C NMR data of the compounds **1** and **2** were listed in Table 1. By compared with the literature, the known compounds were identified as lupinacidin A (**3**),[10] (2*S*)-7,9-dihydroxy-2-(prop-1-en-2-yl)-1,2-dihydroanthra[2,1-*b*]furan-6,11-dione (**4**),[11] austrocortirubin (**5**),[12] 4,6,11-trihydroxy-9-propyltetracene-5,12-dione (**6**),[13] fusarnaphthoquinone B (**7**).[14]

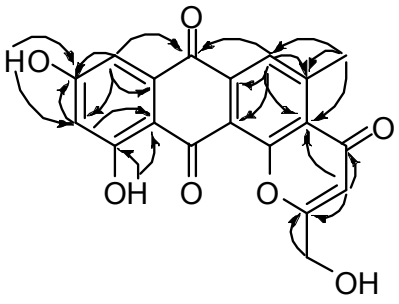

Figure 2. key HMBC ( ) correlations of **1**.

Compound **1** was isolated as a yellow powder. High-resolution ESIMS analysis gave a quasi-molecular ion at *m/z* 351.0501 $[M-H]^-$, consistent with a molecular formula of $C_{19}H_{12}O_7$, which indicated 14 degrees of unsaturation. The UV spectrum of **1** exhibited absorption bands at 364, 276, 254, and 210 nm, highly suggesting the existence of aromatic chromophore.[15] Strong absorption bands accounting for hydroxy (3392 cm^{-1}), carbonyl (1693 and 1650 cm^{-1}), and aromatic

groups (1605, 1564, and 1483 cm^{-1}) could also be observed in its IR spectrum. The ^1H NMR spectrum of **1** (Table 1) showed the presence of two phenolic hydroxy proton (δ_H 11.83 and 12.20), four singlet aromatic protons (δ_H 7.62, 7.07, 6.90, and 6.52), and two aliphatic protons contributed by one methyl singlet (δ_H 2.05), one *O*-methylene singlet (δ_H 4.43). 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 155.0, 161.3, and 164.0), and two carbonyl carbon signals (δ_C 184.4 and 182.0) were observed, which highly suggested the presence of anthraquinone core.[15] The additional carbons account for the remaining substituents, a hydroxymethyl chromone ring (δ_C 182.9 s, 108.2 d, 168.1 s, 62.0 t),[16] and a methyl carbon (δ_C 18.8) on the anthraquinone ring. The substituents and their location on the anthraquinone ring were established by analysis of the HMBC spectra of **1** (Figure 2). The HMBC correlations from a methyl singlet (δ_H 2.05) to C-1 (δ_C 124.0), C-2 (δ_C 144.1), and C-3 (δ_C 133.0) established the location of a methyl at C-2. HMBC correlations between the hydroxy proton (δ_H 12.20) and C-6 (δ_C 161.3), C-7 (δ_C 109.1), and C-5a (δ_C 113.7), as well as those between the other hydroxy proton (δ_H 11.83) and C-7 (δ_C 109.1), C-8 (δ_C 164.0), and C-9 (δ_C 111.0), led to the assignment of the phenolic hydroxy groups at C-6 and C-8. Additionally, H-2′ (δ_H 6.52) showed correlation with the carbon signal of C-3 (δ_C 133.0) 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.

Compounds **2** was also obtained as yellow powder, a molecular formula of $C_{19}H_{12}O_6$ was deduced from the HRESIMS data *m/z* 335.0562 $[M-H]^-$ (calcd *m/z* 335.0556). The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The obvious chemical shift differences resulted from the disappearance of a hydroxymethyl signal δ_C (62.0 t) and δ_H (4.43 s, 2H), and appearance of a methyl group signal δ_C (20.0 q) and δ_H (2.25 s, 3H) in **2.** This indicated that the hydroxymethyl group in **1** was substituted by a methyl group in **2**. The HMBC correlations of the methyl proton signal (δ_H 2.25) with C-2′ (δ_C 108.8) and C-3′ (δ_C 165.4) also indicated the position of methyl group at C-3′. Thus, the structure of **2** was established as 9,11-dihydroxy-

Table 1. ^1H and ^{13}C NMR data of compounds **1** and **2** (δ in ppm, in $CDCl_3$)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*=Hz)	δ_C (m)	δ_H (m, *J*=Hz)
1	124.0 d	7.62, s	124.5 d	7.61, s
2	144.1 s		143.8 s	
3	133.0 s		133.4 s	
4	155.0 s		155.5 s	
4a	117.1 s		117.3 s	
5	184.4 s		183.8 s	
5a	113.7 s		113.6 s	
6	161.3 s		161.8 s	
7	109.1 d	6.90, s	109.1 d	6.80, s
8	164.0 s		163.9 s	
9	111.0 d	7.07 s	110.9 d	7.00 s
9a	122.2 s		122.6 s	
10	182.0 s		182.1 s	
10a	125.4 s		125.2 s	
1′	182.9 s		183.0 s	
2′	108.2 d	6.52, s	108.8 d	6.33, s
3′	168.1 s		165.4 s	
4′	62.0 t	4.43, s	20.0 q	2.25, s
5′	18.8 q	2.05, s	18.3 q	2.01, s
Ar-OH-6		12.20, s		12.22, s
Ar-OH-8		11.83, s		11.78, s

2,5-dimethyl-4*H*-naphtho[2,3-*h*]chromene-4,7,12-trione.

Since certain of the anthraquinones exhibit potential antiviral activities,[17,18] compounds **1-7** were tested for their anti-TMV activities. The inhibitory activities of compounds **1-7** against TMV replication were tested using the half-leaf method.[19] Ningnanmycin, a commercial product for plant disease in China, was used as a positive control. The antiviral inhibition rates of compounds **1-7** at the concentration of 20 μM were listed Table 2. The results showed that compound **7** exhibited high anti-TMV activity with inhibition rate of 35.2%. The inhibition rate is higher than that of positive control. The other compounds also showed potential anti-TMV activities with inhibition rates in the range of 15.2–24.8%, respectively.

Table 2. TMV infection inhibition activities of compounds **1-7**

Compounds	Inhibition rates (%)	Compounds	Inhibition rates (%)
1	24.8 ± 3.6	**5**	17.5 ± 3.1
2	22.4 ±2.8	**6**	21.3 ± 2.4
3	15.2 ± 2.4	**7**	35.2 ± 3.5
4	18.7 ± 2.5	ningnamycin	31.2 ± 3.4

All results are expressed as mean ± SD; n = 3 for all groups.

EXPERIMENTAL

General. 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 mm × 250 mm, 7.0 μm) column and DAD detector.

Plant material. The stems 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. The air-dried and powdered leaves and stems of *C. fistula* (4.8 kg) were extracted four times with 70% acetone (4 × 5 L) at room temperature and filtered. The crude extract (258 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 acetone-$CHCl_3$ (9:1, 8:2, 7:3, 6:4, 1:1), yielded the mixtures A1–A5. The subfraction A1 (9:1, 5.6 g) was subjected to preparative HPLC (65% MeOH, flow rate 12 mL/min) to give **2** (8.57 mg), **3** (12.4 mg), and **4** (22.6 mg). The further separation of subfraction A2 (8:2, 4.8 g) by silica gel column chromatography, and preparative HPLC (60% MeOH,

flow rate 12 mL/min) to give **1** (8.8 mg), **5** (16.4 mg), and **6** (15.8 mg). The further separation of subfraction A3 (7:3, 3.4 g) by silica gel column chromatography, and preparative HPLC (50% MeOH, flow rate 12 mL/min) to give **7** (14.9 mg).

Anti-TMV Assays. The Anti TMV activities were tested using the half-leaf method, and ningnanmycin,[19] a commercial product for plant disease in China, was used as a positive control.

9,11-Dihydroxy-2-(hydroxymethyl)-5-methyl-4*H*-naphtho[2,3-*h*]chromene-4,7,12-trione (**1**): $C_{19}H_{12}O_7$, obtained as yellow powder; UV (MeOH), λ_{max} (log ε) 367 (3.68), 278 (4.20), 255 (3.87), 210 (4.42) nm; IR (KBr) ν_{max} 3392, 2928, 2876, 1693, 1650, 1605, 1564, 1483, 1418, 1362, 1276, 1158, 1134, 1065, 872, 768 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table 1; ESI-MS (negative ion mode) *m/z* 351[M-H]$^-$; HR-ESI-MS (negative ion mode) *m/z* 351.0501 [M-H]$^-$ (calcd 351.0505 for $C_{19}H_{11}O_7$).

9,11-Dihydroxy-2,5-dimethyl-4*H*-naphtho[2,3-*h*]chromene-4,7,12-trione (**2**): $C_{19}H_{12}O_6$, obtained as yellow powder; UV (MeOH), λ_{max} (log ε) 364 (3.71), 276 (4.15), 254 (3.85), 210 (4.38) nm; IR (KBr) ν_{max} 3395, 2926, 2874, 1695, 1652, 1608, 1559, 1487, 1438, 1369, 1274, 1152, 1128, 1057, 883, 764 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table 1; ESI-MS (negative ion mode) *m/z* 335 [M-H]$^-$; HR-ESI-MS (negative ion mode) *m/z* 335.0562 [M-H]$^-$ (calcd 335.0556 for $C_{19}H_{11}O_6$).

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