HETEROCYCLES, Vol. 89, No. 2, 2014, pp. 481 - 486. © 2014 The Japan Institute of Heterocyclic Chemistry
Received, 18th November, 2013, Accepted, 18th December, 2013, Published online, 20th December 2013
DOI: 10.3987/COM-13-12889

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.

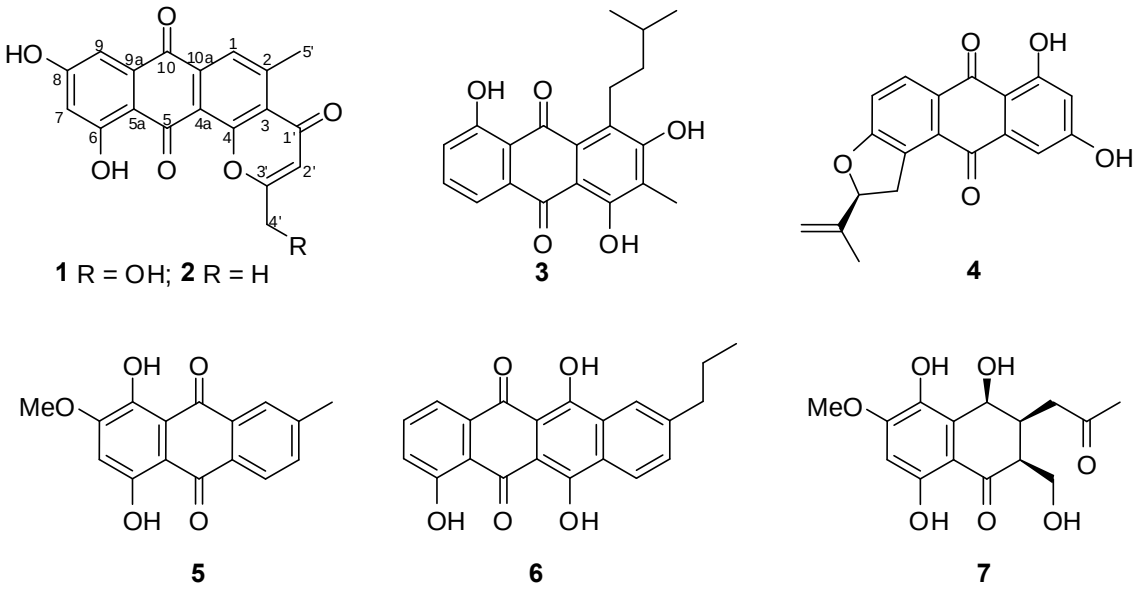

Figure 1. The structures of anthraquinones from *C. fistula*

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]

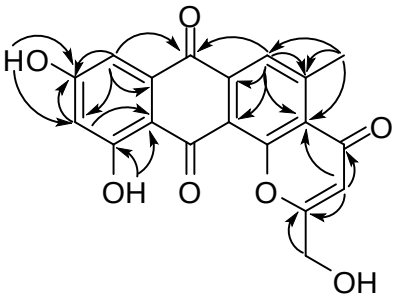

Figure 2. key HMBC ( ) correlations of **1**.

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 $C_{19}H_{11}O_6$).

ACKNOWLEDGMENT

This research was supported by the National Natural Science Foundation of China (No. 21302164), the excellent Scientific and Technological Team of Yunnan High School (2010CI08), the Yunnan University of Nationalities Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008), and Open Research Fund Program of Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University of Nationalities) (2010XY08).

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