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CHROMONES FROM THE TWIGS OF *CASSIA FISTULA* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITIES

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Abstract – Three new chromones, 2-(3-hydroxypropanoyl)-8-methoxy-5-(2-oxopropyl)-4*H*-chromen-4-one (**1**), 8-hydroxy-2-(3-hydroxypropanoyl)-5-(2-oxopropyl)-4*H*-chromen-4-one (**2**), 2-(2-hydroxyethyl)-7-methoxy-5-(2-oxopropyl)-4*H*-chromen-4-one (**3**), together with four known chromones (**3-7**) were isolated from the twigs of *Cassia fistula*. Their structures were determined by means of HRESIMS and extensive 1D and 2D NMR spectroscopic studies. Compounds **1-3** were tested for their anti-tobacco mosaic virus (anti-TMV) activities. The results revealed that compounds **1-3** showed high anti-TMV activities with inhibition rates of 26.6, 28.2 and 29.7%, respectively. These rates are close to that of positive control.

The plant of *Cassia fistula* L., (Leguminosae) belongs to the Cassia genus. It is widely grown as an ornamental plant in tropical and subtropical areas.[1] In China, it also 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,[6,7] chromones,[8-10] flavonoids,[11-13] naphtho[1,2-*b*]furan,[14] and the like. In our continuing efforts to identify bioactive natural products from the medicinal plants, we now investigated the chemical constituents of the twigs of *C. fistula*. This leads to the isolation of three new **(1-3)**, and four known chromones (**4-7**). The structures of **1-7** were elucidated by spectroscopic methods including extensive ^{1}D and ^{2}D NMR techniques. Compounds **1-3** were also evaluated for their anti-tobacco mosaic virus (anti-TMV) activities. This article deals with the

isolation, structural elucidation and biological activities of the new chromones.

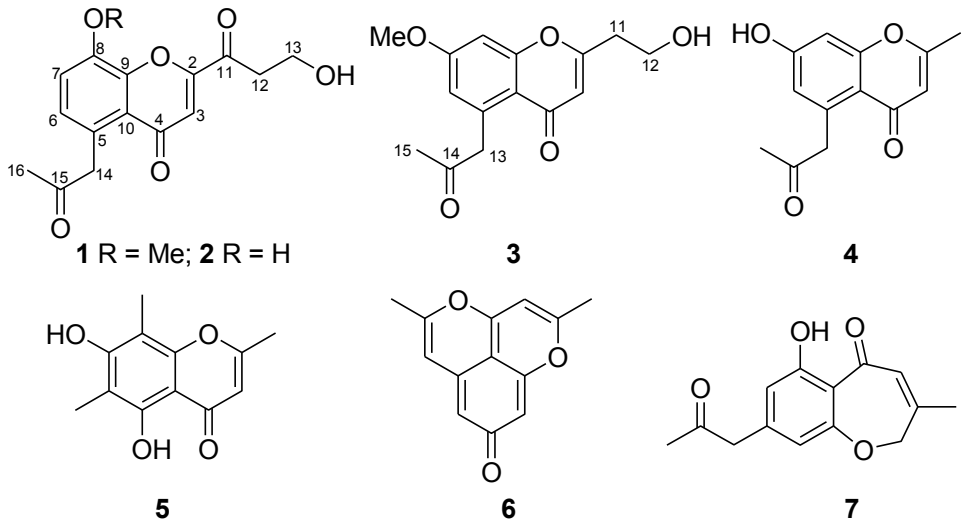

Figure 1. The structures of chromones form the twigs of *C. fistula*

A 70% aq. acetone extract prepared from the twigs of *C. fistula* was subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford three new chromones, 2-(3-hydroxypropanoyl)-8-methoxy-5-(2-oxopropyl)-4*H*-chromen-4-one (**1**), 8-hydroxy-2-(3-hydroxy-propanoyl)-5-(2-oxopropyl)-4*H*-chromen-4-one (**2**), 2-(2-hydroxyethyl)-7-methoxy-5-(2-oxopropyl)-4*H*-chromen-4-one (**3**), and four known chromones (**4**-**7**). The structures of the compounds **1**-**7** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of compounds **1**-**3** were listed in Table 1. The known compounds, compared with literature, were identified as 7-hydroxy-2-methyl-5-(2-oxopropyl)-4*H*-chromen-4-one (**4**),[15] 8-methyleugenitol (**5**),[16] barakol (**6**),[17] and 5-hydroxy-9-methyl-1-(2-oxopropyl)-benzo[*β*]oxepin-7(2*H*)-one (**7**).[18]

Compound **1** was obtained as a yellow gum and assigned the molecular formula $C_{16}H_{16}O_6$ from its HRESIMS at *m/z* 327.0852 $[M+Na]^+$ (calcd 327.0845). The IR absorption bands indicated the presence of hydroxy (3418 cm^{-1}), carbonyl (1730, 1682, 1650 cm^{-1}), and aromatic ring (1610, 1558, 1436 cm^{-1}) groups, and UV absorptions at 210, 238, 272, and 350 nm suggested a conjugated aromatic ring system. Its ^{1}H, ^{13}C, and DEPT NMR spectra displayed signals for 16 carbons and 16 hydrogen atoms, corresponding to one chromone ring system[19] (C-2~C-10) with three aromatic protons (H-3, H-6, and H-7), one 2-oxopropyl moiety (CH_3-CO-CH_2-; C-14~C-16; H-14 and H-16),[20] one 3-hydroxypropanoyl moiety[21] (-CO-CH_2-CH_2-OH; C-11~C-13; H-12 and H-13), and a methoxy group (δ_C 55.9, δ_H 3.83). The HMBC correlations of H-12 (δ_H 3.33) with C-2 (δ_C 156.8) and of H-3 (δ_H 7.12) with C-11 (δ_C 198.1) indicated that the 3-hydroxypropanoyl moiety was located at C-2. The HMBC correlations of H-14 (δ_H 4.18) with C-5 (δ_C 128.3), C-6 (δ_C 125.4), and C-10 (δ_C 120.1) and of H-6 (δ_H 6.81) with C-14 (δ_C 50.2)

indicated that the 2-oxopropyl moiety was attached to C-5. The attachment of the methoxy group at C-8 was supported by the HMBC correlations of the methoxy proton (δ_H 3.83) with C-8 (δ_C 150.8). Thus, the structure of **1** was established as 2-(3-hydroxypropanoyl)-8-methoxy-5-(2-oxopropyl)-4*H*-chromen-4-one.

Compound **2** was obtained as a yellow gum and showed a quasi-molecular ion at *m/z* 313.0680 $[M+Na]^+$ in the HRESIMS (calcd *m/z* 313.0688), corresponding to the molecular formula $C_{15}H_{14}O_6$. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**. The chemical shift differences resulted from the disappearance of a methoxy resonance (δ_C 55.9, δ_H 3.83) and appearance of a phenolic hydroxy proton signal (δ_H 10.60) in **2**. This indicated that the methoxy group at C-8 in **1** was converted into a phenolic hydroxy group in **2**. The HMBC correlations of the phenolic hydroxy proton signal (δ_H 10.60) with C-7 (δ_C 122.1), C-8 (δ_C148.1), and C-9 (δ_C 151.3) indicated that the phenolic hydroxy group was located at C-8. Thus, the structure of **2** was established as 8-hydroxy-2-(3-hydroxypropanoyl)-5-(2-oxopropyl)-4*H*-chromen-4-one.

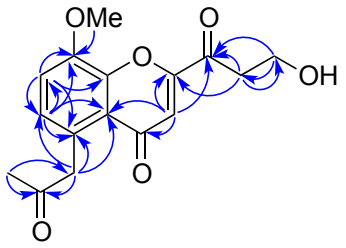

Figure 2. Key HMBC (↷) correlations of **1**

Table 1. ^1H NMR and ^{13}C NMR Data (in $CDCl_3$, 500 and 125 MHz) of compounds **1-3**

NO.	**1**		**2**		**3**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
2	156.8 s		157.0 s		169.0 s	
3	117.0 d	7.12 s	117.2 d	7.14 s	111.8 d	6.21 s
4	180.1 s		179.9 s		181.5 s	
5	128.3 s		129.1 s		138.2 s	
6	125.4 d	6.81 (d) 8.2	126.2 d	6.69 (d) 8.2	117.3 d	6.70 (d) 1.8
7	121.1 d	7.02 (d) 8.2	122.1 d	6.99 (d) 8.2	166.9 s	
8	150.8 s		148.1 s		103.2 d	6.83 (d) 1.8
9	149.6 s		151.3 s		159.2 s	
10	120.1 s		120.4 s		115.2 s	
11	198.1 s		198.2 s		36.2 t	2.60 (t) 7.2
12	42.2 t	3.33 (t) 6.2	42.0 t	3.39 (t) 6.2	62.9 t	3.54 (t) 7.2
13	59.9 t	4.40 (t) 6.2	60.2 t	4.36 (t) 6.2	50.0 t	4.18 s
14	50.2 t	4.18 s	50.0 t	4.16 s	207.8 s	
15	208.2 s		207.8 s		30.8 q	2.30 s
16	30.9 q	2.27 s	30.4 q	2.29 s		
-OMe	55.9 q	3.83 s			56.0 q	3.82 s
Ar-OH				10.60 s		

Compound **3** was also obtained as yellow gums. It had the molecular formula $C_{15}H_{16}O_5$ as revealed by its HRESIMS at *m/z* 299.0897 $[M+Na]^+$ (calcd 299.0895). Its ^1H, ^{13}C, and DEPT NMR spectra (Table 1) displayed signals for 15 carbons and 16 hydrogen atoms, corresponding to one chromone ring system

(C-2~C-10) with three aromatic protons (H-3, H-6, and H-8), one 2-oxopropyl moiety (C-13~C-15; H-13 and H-15), one 2-hydroxyethyl [-CH_2CH_2OH; C-11 and C-12; H-11 and H-12] moiety,[22] and a methoxy group (δ_C 56.0, δ_H 3.82). The HMBC correlations of H-11 with C-2 and C-3, of H-12 with C-2, of H-3 with C-11 indicated that the 2-hydroxyethyl moiety was located at C-2. The HMBC correlations of H-14 with C-5, C-6, and C-10, of H-6 with C-14 indicated that the 2-oxopropyl moiety was attached to C-5. The attachment of the methoxy group at C-7 was supported by the HMBC correlations of the methoxy proton with C-7. The typical proton signals of H-6 (δ_H 6.70, d, J=1.8) and H-8 (δ_H 6.83, d, J=1.8) also supported this substituents pattern. Compound **3** was thus defined as 2-(2-hydroxyethyl)-7-methoxy-5-(2-oxopropyl)-4*H*-chromen-4-one.

Since certain chromones exhibit potential anti-TMV activities,[8,10,18,21] Compounds **1-3** were tested for their anti-TMV activity. The anti-TMV activity was tested using the half-leaf method. Ningnanmycin (a commercial product for plant disease in China) with inhibition rate of 30.8%, was used as a positive control.[23,24] The results revealed that compounds **1-3** showed high anti-TMV activity with inhibition rates of 26.6, 28.2 and 29.7% at the concentration of 20 μM, respectively. These rates are close to that of positive control.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard, and the chemical shifts (δ) were expressed in ppm. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant material. The twigs of *C. fistula* were collected in Dehong prefecture of Yunnan Province, People's Republic of China, in September 2014. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-14-09-35) has been deposited in our Laboratory.

Extraction and Isolation. The air-dried and powdered twigs of *C. fistula* (4.8 kg) were extracted four times with 70% aqueous acetone (3×5 L) at room temperature and filtered. The solvent was evaporated in vacuo, and the crude extract was dissolved in H_2O and partitioned with EtOAc. The EtOAc partition (122 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$–MeOH gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. Further separation of fraction B

(9:1, 18.5 g) by silica gel column chromatography, eluted with $CHCl_3$ –Me_2CO (9:1 - 2:1), yielded mixtures B1–B7. Fraction B3 (7:3, 2.85 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (46% MeOH-H_2O, flow rate 12 mL/min) to give **1** (12.2 mg), **3** (13.4 mg), and **6** (8.2 mg). Fraction B4 (6:4, 4.48 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (38% MeOH-H_2O, flow rate 12 mL/min) to give **2** (11.4 mg) , **4** (15.3 mg), **5** (13.7 mg), and **7** (10.3 mg).

Anti-TMV Assays. The anti-TMV activities were tested using the half-leaf method,[23,24] and Ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as a positive control.

2-(3-Hydroxypropanoyl)-8-methoxy-5-(2-oxopropyl)-4*H*-chromen-4-one (1): Obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.28), 238 (3.81), 272 (3.86), 350 (3.68) nm; IR (KBr) ν_{max} 3418, 3087, 2936, 2854, 1730, 1682, 1650, 1610, 1558, 1436, 1318, 1142, 1057, 953, 876 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; positive ESIMS *m/z* 327 $[M+Na]^+$; positive HRESIMS *m/z* 327.0852 $[M+Na]^+$ (calcd for $C_{16}H_{16}NaO_6$, 327.0845).

8-Hydroxy-2-(3-hydroxypropanoyl)-5-(2-oxopropyl)-4*H*-chromen-4-one (2): Obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.15), 240 (3.86), 275 (3.73), 352 (3.63) nm; IR (KBr) ν_{max} 3452, 3092, 2941, 2850, 1732, 1680, 1653, 1608, 1562, 1435, 1357, 1162, 1049, 918, 852 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; positive ESIMS *m/z* 313 $[M+Na]^+$; positive HRESIMS *m/z* 313.0680 $[M+Na]^+$ (calcd for $C_{15}H_{14}NaO_6$, 313.0688).

2-(2-Hydroxyethyl)-7-methoxy-5-(2-oxopropyl)-4*H*-chromen-4-one (3): Obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.22), 232 (3.65), 265 (3.96), 340 (3.70) nm; IR (KBr) ν_{max} 3423, 2926, 2855, 1720, 1648, 1610, 1552, 1463, 1342, 1135, 1057, 946, 832 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; positive ESIMS *m/z* 299 $[M+Na]^+$; positive HRESIMS *m/z* 299.0897 $[M+Na]^+$ (calcd for $C_{15}H_{16}NaO_5$, 299.0895).

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