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Isoflavanones from the Stem of *Cassia siamea* and Their Anti-tobacco Mosaic Virus Activities

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Two new isoflavanones, (3*R*) 7,2',4'-trihydroxy-3'-methoxy-5-methoxycarbonyl-isoflavanone (**1**) and (3*R*) 7,2'-dihydroxy-3',4'-dimethoxy-5-methoxycarbonyl-isoflavanone (**2**), together with six known isoflavanones (**3**-**8**), were isolated from the stems of *Cassia siamea*. The structure of **1**-**8** was elucidated by spectroscopic methods including extensive 1D- and 2D-NMR techniques. Compounds **1**, **2**, **5**-**8** were evaluated for their anti-tobacco mosaic virus (Anti-TMV) activity. The results showed that compounds **1** and **6** showed potential anti-TMV activity with inhibition rates of 24.6% and 26.9%, respectively. Compounds **2**, **5**, **7**, **8** also showed anti-TMV activity with inhibition rates in the range of 11.8-18.6%.

Key Words : *Cassia siamea*, Isoflavanones, Anti-tobacco mosaic virus activity

Introduction

Cassia siamea Lam. (Fabaceae) belongs to the Cassia genus. It is a terrestrial plant extends in various countries.[1] In China, it has been widely used as traditional Chinese medicine for treatment of diarrhea, gastritis, ringworm, and fungal skin infections.[2,3] Previous phytochemical studies on *C. siamea* has revealed the presence of anthraquinones,[4,5] steroids,[6,7] chromones,[8,9] alkaloids,[10,11] and flavonoids.[12]

In our previous studies, some new chromones[9] and flavonoids[12] possessing anti-tobacco mosaic virus (anti-TMV) and anti-HIV-1 properties were isolated from the stems of *C. siamea* collected in Xishuangbangna Prefecture, Yunnan Province. Motivated by a search for more new bioactive metabolites from this plant, we now investigated the chemical constituents of the stems of *C. siamea* in Dehong Prefecture, Yunnan Province. This lead to the isolation of eight isoflavanones (**1**-**8**), including two new compounds (**1** and **2**). Described in this paper are their structure elucidation and anti-TMV activity.

Results and Discussion

The stems of *C. siamea* were extracted with 70% aqueous acetone. The extract was subjected repeatedly to column chromatography on silica gel, RP-18, and semi-preparative RP-HPLC separation to afford compounds **1**-**8**. 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 identied as (3*S*)-3',7-dihydroxy-2',4',5',8-tetramethoxy-isoavan (**3**),[13] (3*S*)-7-hydroxy-2',3',4',5',8-pentamethoxy-isoavan (**4**),[14] uncinacarpan (**5**),[15] 3,5,7,4'-tetrahydroxy-coumaronochromone (**6**),[16] uncinanone E (**7**),[15] 5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxy isoavanone (**8**).[16]

Compound **1** was obtained as pale yellow gum. The HRESIMS showed the quasi-molecular ion peak at *m/z* 383.0748 $[M+Na]^+$ (calc. for 383.0743, $C_{18}H_{16}NaO_8$), in

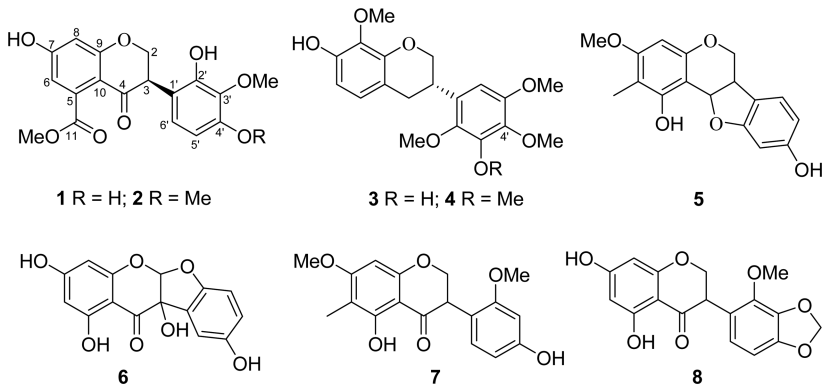

Figure 1. The structure of Isofavanones from the *C. siamea.*

Table 1. ^{1}H and ^{13}C NMR Data of compounds **1** and **2** (δ in ppm, in C_5D_5N, 500 and 125 MHz)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
2	72.2 t	4.49 dd (11.2, 6.4)	72.5 t	4.50 dd (11.2, 6.4)
		4.63 t (11.2)		4.65 t (11.2)
3	47.2 d	4.19 dd (11.2, 6.4)	47.2 d	4.22 dd (11.2, 6.4)
4	197.5 s		197.2 s	
5	136.7 s		136.7 s	
6	107.6 d	6.97 d (2.2)	107.9 d	6.96 d (2.2)
7	165.0 s		164.3 s	
8	104.5 d	6.65 d (2.2)	103.9 d	6.68 d (2.2)
9	158.8 s		158.2 s	
10	108.4 s		108.6 s	
11	169.0 s		168.6 s	
1′	120.4 s		120.0 s	
2′	148.6 s		148.0 s	
3′	139.9 s		138.7 s	
4′	146.2 s		150.9 s	
5′	112.7 d	6.54 d (8.6)	111.7 d	6.62 d (8.6)
6′	121.3 d	6.40 d (8.6)	120.9 d	6.43 d (8.6)
11-OMe	52.5 q	3.95 s	52.9 q	3.95 s
3'-OMe	60.7 q	3.80 s	60.9 q	3.79 s
4'-OMe			55.9 q	3.81 s
7-OH		11.29 s		11.30 s
2'-OH		11.11 s		10.09 s
4'-OH		10.90 s		

accordance with the molecular formula $C_{18}H_{16}O_8$, which indicated 11 degrees of unsaturation. Its UV spectrum showed the maximum absorption at 310, 246, and 210 nm. Strong absorption bands accounting for hydroxy (3382 cm^{-1}), carbonyl (1694, 1652 cm^{-1}), and aromatic groups (1605, 1516, 1462 cm^{-1}) could also be observed in its IR spectrum. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) displayed signals for all 18 carbons and 16 protons, including two aromatic ring (δ_C 136.7, 107.6, 165.0, 104.5, 158.8, 108.4, 120.4, 148.6, 139.9, 146.2, 112.7, 121.3) with four aromatic protons [δ_H 6.97 d (2.2), 6.65 d (2.2), 6.54 d (8.6), 6.40 d (8.6)], one *O*-bearing methylene [δ_C 72.2; δ_H 4.49 dd (11.2, 6.4), 4.63 t (11.2)]; one methane [δ_C 47.2; δ_H 4.19 dd (11.2, 6.4)], one carbonyl group (δ_C 197.5), one methoxycarbonyl group (δ_C 169.0, 52.5; δ_H 3.95 s), one methoxy group (δ_C 60.7; δ_H 3.80 s) and three phenolic hydroxy group (δ_H 11.29 s, 11.11 s, 10.90 s). The proton signals at δ_H 4.63 (1H, t, *J* = 11.2 Hz, H-2a), 4.49 (1H, dd, *J* = 11.2, 6.4 Hz, H-2b), 4.19 (1H, dd, *J* = 11.2, 6.4 Hz, H-3), combined with the carbon signals at δ_C 197.5 (C-4), 72.2 (C-2), 47.2 (C-3) in the ^{13}C-NMR (Table 1), implied compound **1** possessed an isoflavanone skeleton.[17] The HMBC correlation (Figure 2) between the methoxy proton (δ_H 3.80) and C-3' (δ_C 139.9) suggested the methoxy group at C-3'. Three phenolic hydroxy groups located at C-7, C-2', and C-4' were supported by the HMBC correlation of the phenolic hydroxy proton signal (δ_H 11.29) with C-6 (δ_C 107.6), C-7 (δ_C 165.0), C-8 (δ_C 104.5), of (δ_H 11.11) with C-1' (δ_C 120.4), C-2' (δ_C 148.6), C-3' (δ_C 139.9), and of (δ_H 11.90) with C-3' (δ_C 139.9), C-4' (δ_C 146.2), C-5' (δ_C 112.7), respectively. The methoxycarbonyl group at C-5 was supported by HMBC correlations of H-6 (δ_H 6.97) with the ester carbonyl carbon (δ_C 169.0 s), and no correlation was observed between H-8 (δ_H 6.65) and the carbonyl. The typical protons signals (δ_H 6.97, d, *J* = 2.2; 6.65, d, *J* = 2.2; 6.54, d, *J* = 8.6; 6.40, d, *J* = 8.6) also supported the 5,7-disubstituted for ring B, and 2',3',4'-trisubstituted for ring C. The *R* configuration at C-3 was assigned by the comparison of NMR, optical rotation, and CD data with these of known compounds.[17,18] Thus, compound **1** was determined as (3*R*) 7,2',4'-trihydroxy-3'-methoxy-5-methoxycarbonyl-isoflavanone.

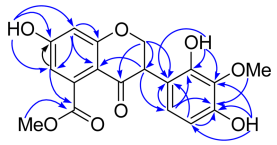

Figure 2. Selected HMBC ( ) correlations of **1**.

Table 2. TMV Infection Inhibition Activities of Compounds **1**, **2**, **5-8**

Compounds		Compounds	
1	24.6 ± 2.7	**7**	11.8 ± 2.0
2	18.6 ± 2.5	**8**	15.6 ± 2.6
5	12.2 ± 1.8	ningnamycin	36.2 ± 3.0
6	26.9 ± 2.2		

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

Compound **2** was also obtained as yellow gum, and should sodiated molecular ions at *m/z* 397.0892 $[M+Na]^+$ in the HRESIMS. The ^{1}H and ^{13}C spectra data of **2** was very similar to these of **1** (see Table 1), except for the appearance of a methoxy signal at (δ_C 55.9; δ_H 3.81) and the disappearance of phenolic hydroxy proton signal at (δ_H 10.90). The HMBC correlations between the methoxy proton signal (δ_H 3.81) and C-4' (δ_C 150.9) suggested the additional methoxy group should be attached at C-4'. This substituent group variation also supported by the NMR data change for the down-shift of C-4' from δ_C 146.2 ppm to δ_C 150.9 ppm. Compound **2** is therefore the 4'-methoxy derivative of **1**.

Since certain of the flavonoids exhibit potential anti-TMV activity.[12,19-21] The compounds **1**, **2**, **5-8** were tested for their anti-TMV activity. The inhibitory activities of compounds **1**, **2**, **5-8** against TMV replication were tested using the half-leaf method.[22] Ningnanmycin, a commercial product for plant disease in China, was used as a positive control. The antiviral inhibition rates of compounds **1**, **2**, **5-8** at the concentration of 20 μM were listed in Table 2. The results showed that compounds **1** and **6** showed potential anti-TMV activity with inhibition rate of 24.6% and 26.9%, respectively. Compounds **2**, **5**, **7**, **8** also showed anti-TMV activity with inhibition rates in the range of 11.8-18.6%.

Experimental Section

General Experimental Procedures. Optical rotations were

measured in a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer and CD spectra were measured on a JASCO J-810 spectropolarimeter. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant Material. The stems of *C. siamea* were collected in Dehong prefecture of Yunnan Province, People's Republic of China, in September 2011. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-11-09-58) has been deposited in our Laboratory.

Extraction and Isolation. The air-dried and powdered *C. siamea* (5.5 kg) were extracted four times with 70% aqueous acetone (4 × 6.0 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (511 g) was decolorized by MCI. The 90% methanol part (180 g) was chromatographed on a silica gel column eluting with a chloroform-acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. The further separation of fraction C (8:2, 22.5 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1–1:2), yielded mixtures C1–C7. Fraction C4 (6:4, 6.21 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (62% MeOH-H_2O, flow rate 12 mL/min) to give **2** (9.26 mg), **3** (12.6 mg), **4** (14.5 mg), and **7** (16.8 mg). Fraction C5 (1:1 6.28 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (55% MeOH-H_2O, flow rate 12 mL/min) to give **1** (15.8 mg), **5** (22.6 mg), **6** (16.5 mg), and **8** (24.7 mg).

Anti-TMV Assays. The Anti TMV activities were tested using the half-leaf method.[22] and Ningnanmycin, a commercial product for plant disease in China, was used as a positive control.

TMV (U1 strain) was obtained from the Key Laboratory of Tobacco Chemistry, Yunnan Academy of Tobacco Science. The virus was multiplied in *Nicotiana tabacum* cv. K326 and purified as previously described.[29] The concentration of TMV was adjusted to 20 mg/mL as determined by UV absorption. [virus concentration = (A_{260} × dilution ratio)/ $E_{1\ cm}^{0.1\%,\ 260\ nm}$]. The purified virus was kept at −20 °C and diluted to 32 μg/mL with 0.01 M PBS before use.

Nicotiana glutinosa plants were cultivated in an insect-free greenhouse. Experiments were conducted when the plants grew to 5- to 6-leaf stage. The tested compounds were dissolved in DMSO and diluted with distilled H_2O to the required concentrations. A solution of equal concentration of DMSO was used as negative control; and ningnanmycin was used as positive control.

For the Half-Leaf Method,[22] the virus was mixed with a solution of the test compound for 30 min before inoculated on the left side of a leaf of *N. glutinosa*, whereas the right side of the leaf was inoculated with a mixture of DMSO and virus as a control. The local lesion numbers were recorded 3-4 days after inoculation. Three leaf blades were used for each compound. The inhibition rates were calculated according to the formula: Inhibition Rate (%) = [(C-T)/C] × 100%, where C is the average number of local lesions in the control and T is the average number of local lesions in the treated leaves.

(3*R*) 7,2',4'-Trihydroxy-3'-methoxy-5-methoxycarbonyl-isoflavanone (1). $C_{18}H_{16}O_8$, pale yellow gum; $[\alpha]_D^{24.6}$ −36.6 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 310 (3.46), 246 (3.28), 210 (4.18) nm; CD (c = 0.2, MeOH) λ_{max} (nm, Δε): 250 (+1.36), 344 (+0.94); IR (KBr): ν_{max} 3382, 2953, 2872, 1694, 1652, 1605, 1516, 1462, 1431, 1468, 1268, 1059, 968, 871 cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz), see Table 1; ESIMS *m/z* 383; HRESIMS *m/z* 383.0748 $[M+Na]^+$ (calcd $C_{18}H_{16}NaO_8$ for 383.0743).

(3*R*) 7,2'-Dihydroxy-3',4'-dimethoxy-5-methoxycarbonyl-isoflavanone (2). $C_{19}H_{18}NaO_8$: Pale yellow gum; $[\alpha]_D^{24.8}$ −39.8 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 312 (3.38), 248 (3.35), 210 (4.22) nm; CD (c = 0.2, MeOH) λ_{max} (nm, Δε): 252 (+1.56), 346 (+1.08); IR (KBr): ν_{max} 3385, 2950, 2874, 1697, 1656, 1602, 1514, 1465, 1432, 1468, 1281, 1055, 973, 869 cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz), see Table 1; ESIMS *m/z* 397; HRESIMS *m/z* 397.0892 $[M+Na]^+$ (calcd $C_{19}H_{18}NaO_8$ for 397.0899).

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