NOTE

A New Phenolic Compound from Stem of *Cassia siamea* and its Anti-Tobacco Mosaic Virus Activity

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A new phenolic compound, 4-(3-hydroxy-4,5-dimethoxyphenyl)-4-oxobutanoic acid (**1**), was isolated from the stem of *Cassia siamea*. Its structure was elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. Compound **1** was also tested for its anti-tobacco mosaic virus activity and it showed high anti-tobacco mosaic virus activity with inhibition rate of 18.6 %.

Keywords: *Cassia siamea*, Phenolic compound, Anti-tobacco mosaic virus activity.

Cassia siamea Lam. (Fabaceae) belongs to the *Cassia* genus. It is a terrestrial plant extends in various countries¹. 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 flavonoids¹².

In our previous studies, some new chromones⁹ and flavonoids¹² possessing anti-tobacco mosaic virus (anti-TMV) and anti-HIV-1 properties were isolated from the stems of *C. siamea* collected in Xishuangbanna 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 leads to the isolation of a new phenolic compound (**1**). Its structure elucidation and anti-TMV activity were described in this paper.

UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininframed spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ¹H, ¹³C 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 separate was used an Agilent 1100 HPLC equipped with ZORBAX-C₁₈ (21.2 mm × 250 mm, 7 mm) column and DAD detector.

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 stem of *C. siamea* (2 kg) were extracted four times with 90 % aqueous ethanol (4 × 3 L) at room temperature and filtered. The crude extract (85 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with gradient CHCl₃-MeOH (9:1, 8:2, 7:3, 6:4, 1:1), to yield mixtures A-E. The further separation of fraction C (5.26 g) by silica gel column chromatography, eluted with CHCl₃-(CH₃)₂CO gradient system (8:2, 7:3, 6:4, 5:5, 3:7, 0:1), gave six subfractions C1-C6. Subfraction C4 (1.28 g) was subjected to preparative HPLC (22 % methanol, flow rate 12 mL/min) to yield **1** (12.6 mg).

4-(3-Hydroxy-4,5-dimethoxyphenyl)-4-oxobutanoic acid (1**):** Obtained as pale yellow oils; UV (MeOH) λ_{max} (log ε) 318 (2.15), 276 (3.82), 210 (4.34) nm; IR (KBr, ν_{max}, cm⁻¹): 3386, 2922, 2834, 1722, 1708, 1625, 1532, 1486, 1434, 1364, 1230, 1168, 1127, 1064, 976, 829; ¹H and ¹³C NMR data (CDCl₃, 500 MHz and 125 MHz), Table-1; positive ESIMS *m/z* 277 [M + Na]⁺; HRESIMS *m/z* 277.0682 [M + Na]⁺ (Calcd. 277.0688 for C₁₂H₁₄NaO₆).

The 90 % aqueous ethanol extract prepared from the stem of *Cassia siamea* was subjected repeatedly to column chromatography on silica gel, RP-18 and Preparative HPLC to afford 4-(3-hydroxy-4,5-dimethoxyphenyl)-4-oxobutanoic acid (**1**).

Compound **1** (Fig. 1) was obtained as pale yellow oils. Its molecular formula was determined as $C_{12}H_{14}O_6$ by HR-ESI-MS m/z 277.0682 $[M + Na]^+$ (Calcd. 277.0688). Its 1H and ^{13}C NMR spectra (Table-1) showed signals of 14 hydrogens and 12 carbons, respectively, corresponding to one tetrasubstituted aromatic ring (δ_C 129.1, 106.5, 153.9, 145.3, 147.0, 113.0) with two aromatic protons (δ_H 6.89 d, $J = 1.8$ Hz; 7.10 d, $J = 1.8$ Hz), two methylene groups (δ_C 38.6 and 32.7; δ_H 3.33 t, $J = 7.4$ Hz and 2.73 t, $J = 7.4$ Hz), one carbonyl group (δ_C 203.0), carboxyl group (δ_C 180.6, δ_H 9.13 brs), two methoxy groups (δ_C 55.7, 60.6; δ_H 3.77 s, 3.80 s) and a phenolic hydroxy group (δ_H 9.84 brs). Strong absorption bands accounting for hydroxy (3386 cm^{-1}), carbonyl (1722 , 1708 cm^{-1}) and aromatic group (1625 , 1532 , 1486 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maximum at 276 nm confirmed the existence of the aromatic function. The 1H - 1H COSY spectrum showed the correlation between H-8 (δ_H 3.33) and H-9 (δ_H 2.73). Both H-8 and H-9 exhibited the HMBC correlations with C-7 (δ_C 203.0) and C-10 (δ_C 180.6). In addition to the HMBC correlations from H-2 (δ_H 6.89) and H-6 (δ_H 7.10) to C-7 (δ_C 203.0) and from H-8 to C-1 (δ_C 129.1), **1** was deduced as 4-oxo-4-phenylbutanoic acid (Ar-C(O)-CH₂-CH₂-COOH) possessing two methoxy groups and one phenolic hydroxy group on the aromatic ring. The HMBC correlations of phenolic hydroxy proton signal (δ_H 9.84) with C-4 (δ_C 145.3), C-5 (δ_C 147.0) and C-6 (δ_C 113.0) indicated that the phenolic hydroxy group should be located at C-5. The HMBC correlations of two methoxy proton signals (δ_H 3.77, 3.80) with C-3 (δ_C 153.9) and C-4 (δ_C 145.3) indicated two methoxy groups should be located at C-3 and C-4, respectively (Fig. 2). Thus, the structure of **1** was established as 4-(3-hydroxy-4,5-dimethoxyphenyl)-4-oxobutanoic acid.

TABLE-1

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

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
1	129.1 s		8	38.6 t	3.33 t (7.4)
2	106.5 d	6.89 d (1.8)	9	32.7 t	2.73 t (7.4)
3	153.9 s		10	180.6 s	9.13 brs
4	145.3 s		-OMe-3	55.7 s	3.77 s
5	147.0 s		-OMe-4	60.6 s	3.80 s
6	113.0 d	7.10 d (1.8)	Ar-OH-5		9.84 brs
7	203.0 s

Fig. 1. Structure of compound **1** Fig. 2. Key HMBC (—) and 1H - 1H COSY (—) correlations of **1**

Since certain of the phenolic compounds exhibit potential anti-TMV activities^{9,13,14}, compound **1** was tested for its anti-TMV activity. The inhibitory activities of compound **1** against TMV replication were tested using the half-leaf method¹⁴. Ningnanmycin, a commercial product for plant disease in China with inhibition rate of 28.8 %, was used as a positive control. Compound **1** showed anti-TMV activity with inhibition rate of 18.6 %.

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