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NOTE

A New Phenolic Amide from Stems of *Cassia fistula* and Their Anti-Tobacco Mosaic Virus Activities

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A new phenolic amide, (E)-3-(3,4-dihydroxyphenyl)-N-[2-(4-methoxyphenyl)-2-oxoethyl]-prop-2-enamide (**1**) was isolated from the stems of *Cassia fistula*. Its structure was elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. Compound **1** was also evaluated for its anti-tobacco mosaic virus (anti-TMV) activity and it exhibit potential anti-TMV activity with inhibition rates of 16.2 %.

Keywords: *Cassia fistula*, Phenolic amide, Anti-tobacco mosaic virus activity.

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]. Motivated by a search for new bioactive metabolites from this plant, our research group has investigated the chemical constituents of the stem of *C. fistula*, which led to the isolation and characterization of a new phenolic amide. This paper deals with the isolation, structural characterization and the anti-tobacco mosaic virus (anti-TMV) activity of this compound.

UV spectra were obtained on 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 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, P.R. 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.

The stems of *C. siamea* were collected in Honghe prefecture of Yunnan Province, People's Republic of China, in September 2012. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-12-09-64) has been deposited in our Laboratory.

Extraction and isolation: The air-dried and powdered *C. siamea* (2.2 kg) were extracted four times with methanol (4 × 3 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure and the crude extract (63.5 g) was decolorized by MCI. The 90 % methanol part (31.2 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, 5.47 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1-1:2), yielded mixtures C1-C6. Fraction C3 (7:3, 0.82 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (48 % MeOH-H_2O, flow rate 12 mL/min) to give **1** (8.5 mg).

(*E*)-3-(3,4-Dihydroxyphenyl)-*N*-[2-(4-methoxyphenyl)-2-oxoethyl]-prop-2-enamide (1): Obtained as white powder; IR (KBr, ν_{max}, cm^{-1}): 3410, 3312, 2915, 2586, 1675, 1638, 1527, 1455, 1232, 1168, 1121, 862, 793; UV (MeOH), λ_{max} (log ε): 215 (4.20), 245 (2.56), 292 (4.08), 322 (3.46); ^{1}H and

^{13}C NMR spectroscopic data (500 and 125 MHz, $CDCl_3$), see Table-1; ESI-MS (positive mode) *m/z* 350 $[M + Na]^+$; HRESIMS (positive mode) *m/z* 350.1011 $[M + Na]^+$ (Calcd. for $C_{18}H_{17}NNaO_5$, 350.1004).

The stems of *C. siamea* were extracted with methanol. The extract was subjected repeatedly to column chromatography on silica gel, RP-18 and semi-preparative RP-HPLC separation to afford compound **1**. Its structure was shown in Fig. **1**. The ^{1}H and ^{13}C NMR data of the compound **1** was listed in Table-1.

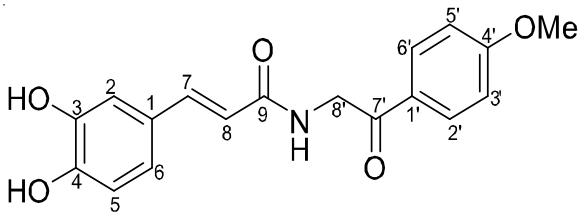

Fig. 1. Structure of compound **1**

Table-1
^{1}H AND ^{13}C NMR DATA OF COMPOUND **1**
(δ in ppm, in $CDCl_3$, 500 AND 125 MHz)

No.	δ_C	δ_H Mult., (*J* Hz)	No.	δ_C	δ_H Mult., (*J* Hz)
1	127.5 s		2',6'	130.3 d	7.90 d (8.8)
2	114.8 d	6.92 d (1.8)	3',5'	115.2 d	6.76 d (8.8)
3	145.6 s		4'	163.8 s	
4	148.2 s		7'	193.3 s	
5	116.6 d	6.78 d (8.6)	8'	45.6 t	4.65 d (5.8)
6	121.5 d	6.85 d (8.6, 1.8)	-NH		8.29 t (5.8)
7	140.8 d	7.25 d (16.0)	-OMe	55.9 q	3.76 s
8	118.2 d	6.52 d (16.0)	Ar-OH-3		9.62 s
9	166.5 s		Ar-OH-4		9.53 s
1'	127.6 s				

Compound (**1**) was isolated as a white powder. Its molecular formula $C_{18}H_{17}NO_5$ was determined from the quasi-molecular ion peak observed using electrospray ionization (ESI)-MS and HRESI-MS measurement at *m/z* 350.1011 $[M + Na]^+$ (Calcd. for $C_{18}H_{17}NNaO_5$, 350.1004), suggesting a 11 degrees of unsaturation. Strong absorption bands accounting for hydroxy (3410 cm^{-1}), amino (3312 cm^{-1}), carbonyl (1675 cm^{-1}) and aromatic group (1638, 1527, 1455 cm^{-1}) could be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 215, 245, 292 and 322 confirmed the existence of the aromatic function. The ^{1}H and ^{13}C NMR spectrum of **1** revealed an caffeic acid moiety (C-1- C-9; H-2, H-5, H-6, H-7 and H-8) and an 2-amino-1-(4-methoxyphenyl) ethanone moiety (C-1'-C-8'; H-2',6', H-3',5', H-8' and NH) (Table-1). The NMR data of **1** were similar to those of tribulusamide D[10], the major difference being the replacement of a hydroxy proton signal in tribulusamide D by a methoxy signal (δ_C 55.9, δ_H 3.76) in **1**. The HMBC correlation (Fig. 2) of the methoxy proton (δ_H 3.76) with C-4' (δ_C 163.8) indicated that the methoxy group was located at C-4'. Compound **1** is therefore the 4'-methoxy derivative of tribulusamide D.

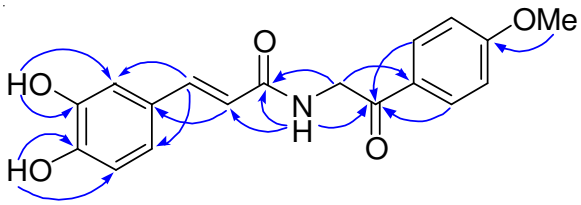

Fig. 2. HMBC correlations ( ) of **1**

Since certain of the phenolic compounds exhibit potential anti-TMV activities[11-13], compound **1** was tested for its anti-TMV activity. The inhibitory activity of compound **1** against TMV replication were tested using the half-leaf method[12,13]. Ningnanmycin (a commercial product for plant disease in China, with inhibition rate of 29.5 %) was used as a positive control. The result showed that compound **1** exhibited potential anti-TMV activity with inhibition rate in the range of 16.2 %.

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