NOTE

A New Chromone from the Stems of *Cassia fistula* and its Anti-Tobacco Mosaic Virus Activity

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A new chromone *i.e.*, 6-methoxy-3-methyl-8-(2-oxopropyl)benzo[b]oxepin-5(2*H*)-one (**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 activity, and it exhibit potential anti-tobacco mosaic virus activity with inhibition rate of 22.5 %.

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

Cassia fistula L., (Leguminosae) is an ornamental tree with beautiful yellow flowers¹. In China, it has been used as traditional Chinese medicine by people of Dai nationality^{2,3}. Previous phytochemical studies of *C. fistula* have shown the presence of anthraquinones^{4,5}, steroids⁶, chromones^{7,8} and flavonoids⁹. Motivated by a search for new bioactive metabolites from this plant, our group has reinvestigated the chemical constituents of the stem of *C. fistula*, which led to the isolation and characterization of a new chromone. 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 $\times$ 25 cm) or Venusil MP C₁₈ (20 mm $\times$ 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 μ m, Merck, Darmstadt, Germany) and MCI gel (75-150 μ m, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 5 % H₂SO₄ in EtOH.

The stems of *C. siamea* were collected in Xishuangbanna 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-12-09-65) has been deposited in our Laboratory.

Extraction and isolation: The air-dried and powdered *C. siamea* (1.8 kg) were extracted four times with methanol (4 $\times$ 3 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure and the crude extract (48.6 g) was decolorized by MCI. The 90 % methanol part (28.5 g) was chromatographed on a silica gel column eluting with a CHCl₃-MeOH (9:1, 8:2, 7:3, 6:4, 1:1), to yield mixtures A-E. The further separation of fraction A (9:1, 8.54 g) by silica gel column chromatography, eluted with CHCl₃-(CH₃)₂CO gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), gave six fractions A1-A6. Subfraction A2 (9:1, 1.05 g) was subjected to preparative HPLC (60 % methanol, flow rate 12 mL/min) to give **1** (8.5 mg).

6-Methoxy-3-methyl-8-(2-oxopropyl)benzo[b]oxepin-5(2*H*)-one (1**):** Obtained as pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 210 (4.28), 255 (3.72), 296 (3.51) nm; IR (KBr, ν_{max} , cm⁻¹): 3432, 2915, 2864, 1702, 1645, 1608, 1572, 1462, 1386, 1208, 1145, 973, 858; ¹H and ¹³C NMR data (CDCl₃, 500 and 125 MHz), in Table-1. positive ESIMS m/z 283 [M + Na]⁺; negative HRESIMS m/z 283.0940 [M - H]⁻ (Calcd. for C₁₅H₁₆NaO₄, 283.0946).

The stems of *C. siamea* were extracted with methanol. The extract was subjected repeatedly to column chromatography on silica gel and preparative RP-HPLC separation to

afford compound **1**. Its structure was shown in Fig. 1. The ^1H NMR and ^{13}C NMR data of the compound **1** was listed in Table-1.

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

TABLE-1
 ^1H AND ^{13}C NMR DATA OF COMPOUND **1**
(500 AND 125 MHz, IN CDCl_3)

No.	δ_{C} (m)	δ_{H} (m, J, Hz)	No.	δ_{C} (m)	δ_{H} (m, J, Hz)
1	143.8 s		9	154.8 s	
2	111.4 d	6.56 (d) 1.8	10	72.5 t	4.48 s
3	160.2 s		11	22.8 q	2.05 s
4	112.8 s		1'	50.5 t	4.12 s
5	166.2 s		2'	205.3 s	
6	114.1 d	6.67 (d) 1.8	3'	30.2 q	2.46 s
7	193.2 s		5-OMe	55.9 q	3.78 s
8	128.4 d	6.32 s			

Compound **1** was obtained as pale yellow oil and was assigned the molecular formula of $\text{C}_{15}\text{H}_{16}\text{O}_4$ by HRESIMS at m/z 283.0940 $[\text{M} + \text{Na}]^+$ (Calcd. m/z 283.0946). The IR showed hydroxyl group at 3432, carbonyl group at 1702 and 1645 and aromatic group at 1608, 1572, 1462 cm^{-1} . UV spectrum of **1** also exhibited absorption bands for aromatic ring at 296 and 255 nm. The ^{13}C NMR spectrum of **1** (Table-1) contained 15 carbons, including eight olefinic (δ_{C} 143.8 s, 111.4 d, 160.2 s, 112.8 s, 166.2 s, 114.1 d, 128.4 d and 154.8 s) carbons, two carbonyl (δ_{C} 193.2 s and 205.3 s) carbons, two methyl (δ_{C} 22.8 q and 30.2 q) carbons, two methylene (δ_{C} 72.5 t and 50.5 t) carbons and one methoxy (δ_{C} 55.9) carbons. The ^1H NMR spectrum of **1** also showed signals of three aromatic ring protons (δ_{H} 6.56 (d) 1.8, 6.67 (d) 1.8 and 6.32 s), two singlet methyl protons (δ_{H} 2.05 s and 2.46 s), two singlet methylene protons (δ_{H} 4.12 s and 4.48 s) and a methoxy proton (δ_{H} 3.78 s). These signals suggested compound **1** containing a 1,5-substituted, 9-methylbenzo[β]oxepin-7(2H)-one moiety¹⁰, an 2-oxopropyl group¹¹, and a methoxy group. The HMBC correlations (Fig. 2) of H-8 (δ_{H} 6.32) with C-4 (δ_{C} 112.8), C-7 (δ_{C} 193.2), C-10 (δ_{C} 72.5) and C-11 (δ_{C} 22.8), of H-10 (δ_{H} 4.48) with C-3 (δ_{C} 160.2) and C-8 (δ_{C} 128.4), of H-11 (δ_{H} 2.05) with C-8 (δ_{C} 128.4), C-9 (δ_{C} 154.8) and C-10 (δ_{C} 72.5), also support the existence of the 9-methylbenzo[β]oxepin-7(2H)-one moiety. The long-range correlations between H-1' (δ_{H} 4.12) and C-1 (δ_{C} 143.8), C-2 (δ_{C} 111.4) and C-6 (δ_{C} 114.1), demonstrates the 2-oxopropyl moiety located at C-1. More-

over, the HMBC correlations between methoxy proton (δ_{H} 3.78) and the signal of C-5 (166.2) confirmed the methoxy group at C-5. Thus, the structure of **1** was established as 6-methoxy-3-methyl-8-(2-oxopropyl)benzo[β]oxepin-5(2H)-one.

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

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 31.5 %) was used as a positive control. The result showed that compound **1** exhibited potential anti-TMV activity with inhibition rate in the range of 22.5 %.

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