Asian Journal of Chemistry; Vol. 25, No. 12 (2013), 6622-6624

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14399

# A New Flavonoid from the Roots of *Cassia fistula* and Its Antitobacco Mosaic Virus Activity

XIAOYING ZENG[1,2], WEI ZHAO[2], TAO ZHANG[2], LAN WANG[1], GUANGYU YANG[2], YONG-KUAN CHEN[2], QIUFEN HU[3,*] and MINGMING MIAO[1,2,*]

[1]Technology Center of Hongyun and Honghe (Group) Co., Ltd., Kunming 650202, P.R. China
[2]Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P.R. China
[3]Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University of Nationalities), State Ethnic Affairs Commission & Ministry of Education, Kunming 650031, P.R. China

*Corresponding authors: E-mail: mmmiao@cyats.com; huqiufena@yahoo.com.cn

(*Received*: 7 August 2012; *Accepted*: 24 May 2013) AJC-13535

A new flavonoid, fistulaflavonoid A (**1**), was isolated from the roots of *Cassia fistula*. Its structure was elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compound **1** was tested for their antitobacco mosaic virus activity. The compound shows modest antitobacco mosaic virus activity with inhibition rates of 18.2 %.

**Key Words: Flavonoid, *Cassia fistula*, Antitobacco mosaic virus.**

## INTRODUCTION

*Cassia fistula* L., (Leguminosae) is an ornamental tree with beautiful yellow flowers. This plant can be found in various countries in Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil[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 of *C. fistula* have shown the presence of anthraquinones[4,5], steroids[6], chromones[7,8] and flavonol derivatives[9]. Motivated by a search for new bioactive metabolites from this plant, our group has investigated the chemical constituents of the roots of *C. fistula*, which led to the isolation and characterization of a new flavonoid. Its structure was established by means of spectroscopic methods including extensive 1D and 2D NMR techniques. This paper deals with the isolation, structural characterization and the antitobacco mosaic virus activity of the new compound.

## EXPERIMENTAL

Optical rotation was measured in Horiba SEPA-300 high sensitive polarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. $^{1}H$, $^{13}C$ 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). Preparative HPLC was used an Agilent 1100 HPLC equipped with ZORBAX-$C_{18}$ (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

The roots of *Cassia fistula* L., (Leguminosae) were collected on Xishuangbangna Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Dr. Yuan. N, of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (YNNU 10-9-28) has been deposited in our Laboratory.

**Extraction and isolation:** The air-dried and powdered roots of *C. fistula*. (2.2 kg) were extracted four times with 70 % methanol (4 × 2.0 L) at room temperature and filtered. The crude extract (115 g) was applied to silica gel (200-300 mesh) column chromatography, 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 D (7:3, 16.4 g) by silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures D1-D5. Fraction D2 (9:1, 2.26 g) was subjected to preparative HPLC (38 % methanol, flow rate 12 mL/min) to give **1** (17.5 mg).

**Fistulaflavonoid A:** Obtained as pale yellow gum; $[\alpha]^{D}_{22.5}$ + 27.5 (c 0.020, MeOH); UV (MeOH), $\lambda_{max}$ (log ε) 348 (2.18), 298 (4.05), 210 (4.74) nm; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3382, 2962, 2870, 1619, 1538, 1482, 1420, 1370, 1250, 1162, 1047, 864; $^{1}H$ and $^{13}C$ NMR data ($C_5D_5N$, 500 and 125 MHz), Table-1; ESI-MS (positive ion mode) m/z 339 $[M+Na]^{+}$; HR-ESI-MS

TABLE-1
$^1$H NMR AND $^{13}$C NMR DATA OF COMPOUND **1** IN $CD_3OD$

| No. | Compound **1** | |
|---|---|---|
| | $\delta_C$ (mult.) | $\delta_H$ (mult, *J*, Hz) |
| 2α | 67.2, t | 4.28, dd, *J* = 4.5, 10.5 |
| 2β | – | 3.50, t, *J* = 10.5 |
| 3 | 41.8, d | 3.44, m |
| 4 | 80.2, d | 5.42, d, *J* = 6.6 |
| 5 | 122.8, d | 6.92, d, *J* = 8.4 |
| 6 | 110.0, d | 6.55, d, *J* = 8.4 |
| 7 | 149.8, s | – |
| 8 | 133.6, s | – |
| 9 | 146.2, s | – |
| 10 | 114.2, s | – |
| 1' | 122.2, s | – |
| 2' | 149.1, s | – |
| 3' | 132.8, s | – |
| 4' | 150.2, s | – |
| 5' | 105.9, d | 6.43, d, *J* = 8.1 |
| 6' | 115.8, d | 6.68, d, *J* = 8.1 |
| 7-OMe | 55.8, q | 3.79 (s) |
| 4'-OMe | 55.9, q | 3.81 (s) |

(positive ion mode) m/z 339.0840 $[M + Na]^+$ (calcd. (%) 339.0845 for $C_{17}H_{16}NaO_6$).

## RESULTS AND DISCUSSION

A 70 % aq. methanol extract prepared from the roots of *C. fistula* was subjected repeatedly to column chromatography and preparative HPLC to afford compound **1**. Its structure was shown in Fig. 1 and its $^1$H and $^{13}$C NMR spectroscopic data were listed in Table-1.

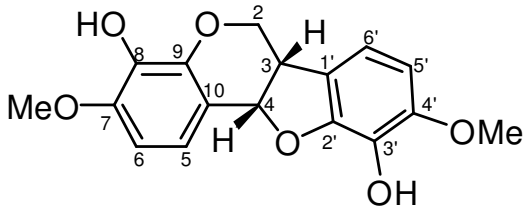


Fig. 1. Structure of new flavonoid

Compound **1** was obtained as a pale yellow gum with $[\alpha]^{D}_{22.5}$ + 27.5 (c 0.020, MeOH). The absorption bands accounting for hydroxyl (3382 $cm^{-1}$) and aromatic groups (1619, 1538, 1482, 1420 $cm^{-1}$) could be observed in its IR spectrum. Its molecular formula was established by the positive mode HRESIMS (high-resolution electronspray ionization mass spectra) peak at m/z 339.0840 $[M + Na]^+$ (calcd. (%) 339.0845 for $C_{17}H_{16}NaO_6$). The $^1$H NMR spectrum suggested a pterocarpan structure due to the splitting pattern of the protons at $\delta_H$ 4.28 (dd, *J* = 4.5, 10.5 Hz, H-2α), $\delta_H$ 3.50 (t, *J* = 10.5 Hz, H-2β), $\delta_H$ 3.44 (m, H-3) and $\delta_H$ 5.42 (d, *J* = 6.6 Hz, H-4), related to the protons of the heterocyclic ring B. This spectrum also allowed the identification of two pairs of ortho situated aromatic protons at $\delta_H$ 6.92 (d, *J* = 8.4 Hz, H-5), $\delta_H$ 6.55 (d, *J* = 8.4 Hz, H-6), $\delta_H$ 6.43 (d, *J* = 8.1 Hz, H-5') and $\delta_H$ 6.68 (d, *J* = 8.1 Hz, H-6') and gave a clear evidence of the 7,8,3',4'-substitution pattern of the pterocarpan moiety. In addition, the presence of two methoxyl groups at $\delta_H$ 3.79 (s) and $\delta_H$ 3.81 (s) was consistent with a dihydroxylated pterocarpan skeleton. All these data were supported by the $^{13}$C and DEPT spectrum that revealed 17 carbon atoms corresponding to two methoxyl, one methylene, 6 methines and 8 non-hydrogenated carbons. However, the confirmation of the above suggestion for **1** was supported by the HSQC and HMBC (Fig. 2) experiments, which allowed the unequivocal assignments of its $^{13}$C and $^1$H NMR data. The assignments of the relative positions of the methoxyl and the hydroxyl groups at C-3' and C-4', respectively, were defined in the HMBC spectrum that showed cross-peaks of the methyl protons at $\delta_H$ 3.81 (-OMe) and the carbon at $\delta_C$ 150.2 (C-4'). Moreover, the correlation of hydrogen at $\delta_H$ 6.92 (H-5) with the carbons at $\delta_C$ 146.2 (C-9), $\delta_C$ 80.2 (C-4) and $\delta_C$ 149.8 (C-7), the correlations between the hydrogen at $\delta_H$ 6.55 (H-8) with the carbons at $\delta_C$ 149.8 (C-7), $\delta_C$ 114.2 (C-10) and the correlations between the hydrogen at $\delta_H$ 3.79 (-OMe) with the carbons at $\delta_C$ 149.8 (C-7), definitively established that the other methoxyl group was located at the C-7 and hydroxyl group was located at the C-8 in the tetrassubstituted aromatic A-ring. It is well known from the literature that, according to biogenetical regulations, the hydrogens (H-3 and H-4) at the B/C rings junction of all natural pterocarpans are always *cis*, either α, α or β, β, thus leading to only two enantiomeric forms. It is also known, through CD (circular dichroism) and/or ORD (optical rotatory dispersion) analyses, that (–) optical rotation can be associated with α, α positioning (3R, 4R), while the (+) optical rotation can be associated with the β, β positioning (3S, 4S) of both series[10,11]. From the (+) optical rotation of compound **1**, it could be assumed an (3S, 4S) absolute configuration for it. As expected, the CD spectrum of **1** should a similar profile of that from (+)-pterocarpin and almost a mirror image of (-)-maackiain, what is in agreement with the suggested (3S, 4S) absolute stereochemistry for compound **1**. Thus, the structure of **1** was determined and named as fistulaflavonoid A.

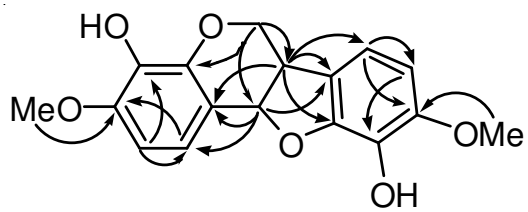


Fig. 2. Selected HMBC (↷) correlations of **1**

Since several flavonoids exhibited anti virus activities[12,13], compound **1** was tested for its antitobacco mosaic virus activity.

The anti tobacco mosaic virus activities were tested using the half-leaf method[14]. The inhibitory activities of the new compound (at the concentration of 20 mM) against tobacco mosaic virus replication were tested using two approaches. First, the half-leaf method was used to test the antiviral activity in the local lesion host *N. glutinosa in vivo*. Then, the leaf-disk method was used to evaluate the antiviral activity of the compound in the systemic infection host *N. tabacum* cv. K326. Ningnanmycin (2 % water solution), a commercial product for plant disease in China, was used as a positive control. The results showed that the compound **1** exhibited inhibition rates of 18.2 %.

## ACKNOWLEDGEMENTS

This project was supported financially by the Basic Research Foundation of Yunnan Tobacco Industry Co. Ltd. (2012JC01), the Excellent Scientific and Technological Team of Yunnan High School (2010CI08), and the Open Research Fund Program of Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University of Nationalities) (2010XY08).

## REFERENCES

1. V. Duraipandiyan and S. Ignacimuthu, *J. Ethnopharmacol*., **112**, 590 (2007).
2. S. Rajan, D.S. Baburaj, M. Sethuraman and S. Parimala, *Ethnobotany*, **6**, 19 (2001).
3. J. Ma, L.X. Zhang and Y.H. Guan, *Chin. J. Ethnomed. Ethnopharm*., **5**, 178 (2004).
4. K.A. Abo, A.A. Adeyemi and A.O. Sobowale, *Afr. J. Med. Med. Sci*., **30**, 9 (2001).
5. S. Aurapa and G. Wandee, *Int. J. Biomed. Pharm. Sci*., **3**, 42 (2009).
6. P. Sartorelli, S.P. Andrade, M.S. Melhem, F.O. Prado and A.G. Tempone, *Phytother. Res*., **21**, 644 (2007).
7. Y.H. Kuo, P.H. Lee and Y.S. Wein, *J. Nat. Prod*., **65**, 1165 (2002).
8. Z. Zuraini, C. Yeng, L.L. Yee, Y.L. Lachimanan, N.S. Lai and S. Sreenivasan, *Molecules*, **16**, 7583 (2011).
9. B. Theeshan, S.N. Vidushi and I.A. Okezie, *Afr. J. Biotechnol.*, **4**, 1530 (2005).
10. T. Miyase, A. Ohtsubo, A. Ueno, T. Noro, M. Kuroyanagi and S. Fukushima, *Chem. Pharm. Bull*., **30**, 1986 (1982).
11. S. Antus, T. Kurtan, L. Juhasz, L. Kiss, M. Hollosi and S. Majer, *Chirality*, **13**, 493 (2001).
12. Y.K. Chen, X.S. Li, G.Y. Yang, Z.Y. Chen, Q.F. Hu and M.M. Miao, *J. Asian Nat. Prod. Res*., **14**, 450 (2012).
13. Z.Y. Chen, J.N. Tan, G.Y. Yang, M.M. Miao, Y.K. Chen and T.F. Li, *Phytochem. Lett*., **5**, 233 (2012).
14. X.H. Yan, J. Chen, Y.T. Di, X. Fang, J.H. Dong, P. Sang, Y.H. Wang, H.P. He, Z.K. Zhang and X.J. Hao, *J. Agric. Food. Chem*., **58**, 1572 (2010).