HETEROCYCLES, Vol. 92, No. 1, 2016, pp. 114 - 119. © 2016 The Japan Institute of Heterocyclic Chemistry
Received, 8th November, 2015, Accepted, 11th December, 2015, Published online, 18th December, 2015
DOI: 10.3987/COM-15-13361

ISOQUINOLINE ALKALOIDS FROM THE TWIGS OF *CASSIA FISTULA* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITY

Hai-Yan Wu,[1] Qiang Liu,[2] Zhen-Hua Yu,[2] Wei-Yao Hu,[2] Ke-Liang Yan,[2] Ye-De Wang,[1] Kun Zhou,[1] Wei Dong,[1] Min Zhou,[1,*] and Qiu-Fen Hu[1,*]

[1] Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University, Kunming 650031, P. R. China; [2] Research and Development of Center, China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, P. R. China; E-mail: zhouminynun@163.com; huqiufena@aliyun.com

Abstract – Two isoquinoline alkaloids, including a new natural product (**1**) and a compound (**2**) isolated from plant for the first time, and named fistulatins A and B (**1** and **2**), together with four known isoquinoline alkaloids (**3**-**6**) were isolated from the twigs of *Cassia fistula*. Their structures were determined by means of HRESIMS and extensive 1D and 2D NMR spectroscopic studies. Compounds **1**-**6** were tested for their anti-tobacco mosaic virus (anti-TMV) activity. The results showed that all isolates showed weak anti-TMV activity with inhibition rates in the range of 15.4-23.5%.

Cassia fistula (Caesalpiniaceae) is native to India, and it is now widely grown as an ornamental plant in tropical and subtropical areas. Its barks contain tannin, a source of red dye. The fruit pulp and seeds are used medicinally as a laxative.[1] Its roots, twigs, barks, seeds, and leaves are used to treat some diseases, such as relieving internal heat, inducing diuresis for removing edema by Dai people in Yunnan Province of China.[2,3] Previous phytochemical investigations revealed that *C. fistula* was a rich source of anthraquinones,[4,5] steroids,[6,7] chromones,[8-10] flavonoids,[11-13] alkaloids,[14,15] naphtho[1,2-*b*]furan,[16] and so on. For the purpose of further utilizing *C. fistula* and identifying more bioactive natural products from this plants, a study of the twigs of *C. fistula* was undertaken and lead to the isolation of a new natural product (**1**),[17] a new compound **(2)**, and four known isoquinoline alkaloids (**4**-**6**). All compounds were isolated from this plant for the first time. The structures of **1**-**6** were elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Compounds **1**-**6** were also evaluated for their anti-tobacco mosaic virus (anti-TMV) activity. The isolation, structural elucidation, and anti-TMV

activity of these compounds are described in this manuscript.

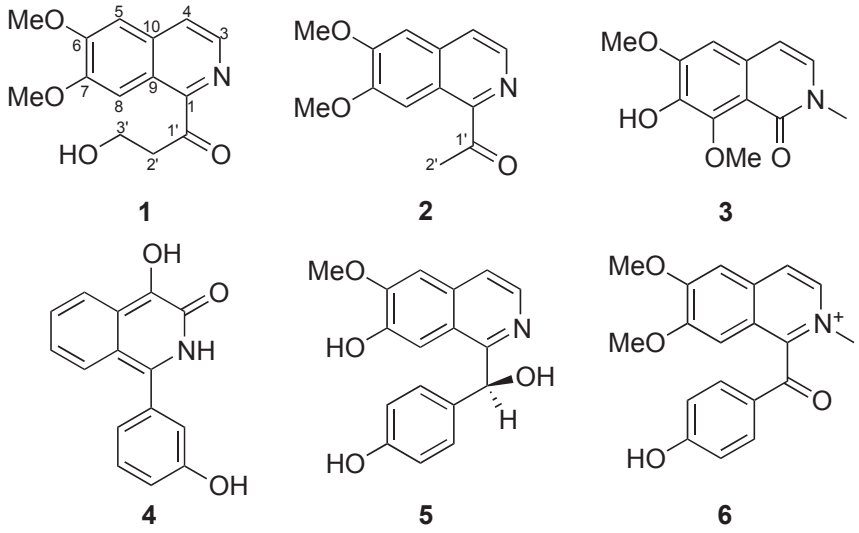


Figure 1. The structures of isoquinoline alkaloids from the twigs of *C. fistula*

A 70% aq. acetone extract prepared from the twigs of *C. fistula* was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9 with saturated Na_2CO_3 aq. and extracted with EtOAc. The EtOAc-soluble alkaloidal materials was subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford two new natural isoquinoline alkaloids, fistulatins A and B (**1** and **2**), and four known isoquinoline alkaloids (**3**-**6**). The structures of the compounds **1**-**6** were as shown in Figure 1, and the ^1H and ^{13}C NMR data of compounds **1** and **2** were listed in Table 1. The known compounds, according to compare with literature, were identified as cherianoine (**3**),[18] phomopsin A (**4**),[19] annocherine A (**5**),[18] and gandharamine (**6**).[20]

Compound **1** was obtained as a yellow amorphous powder and assigned the molecular formula $C_{14}H_{15}NO_4$ from its HRESIMS at *m/z* 284.0889 $[M+Na]^+$ (calcd 284.0899). The IR absorption bands indicated the presence of hydroxy (3418 cm^{-1}), carbonyl (1657 cm^{-1}), and aromatic ring (1622, 1568, 1455 cm^{-1}) groups. UV absorptions at 215, 262, 298, and 336 nm suggested a conjugated aromatic ring system. Its ^1H, ^{13}C, and DEPT NMR data displayed resonances for 14 carbons and 15 hydrogen atoms, corresponding to one 1,6,7-substituted isoquinoline system[18,20] (C-1~C-10; H-3, H-4, H-5, and H-8), one 3-hydroxypropanoyl moiety[21] (-CO-CH_2-CH_2-OH; C-1′~C-3′; H_2-2′ and H_2-3′), and two methoxy group (δ_C 56.0 q and 56.2 q; δ_H 3.78 s, 3.83 s). The existence of isoquinoline system was also supported by the HMBC correlations (Figure 2) of H-3 with C-1, C-4, and C-10, of H-4 with C-3, C-9, and C-10, of H-5 with C-4, C-9, C-10, and of H-8 with C-1, C-9, and C-10. The 3-hydroxypropanoyl moiety located at C-1

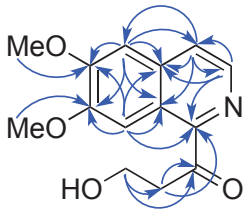


Figure 2. Key HMBC (↷) correlations of **1**

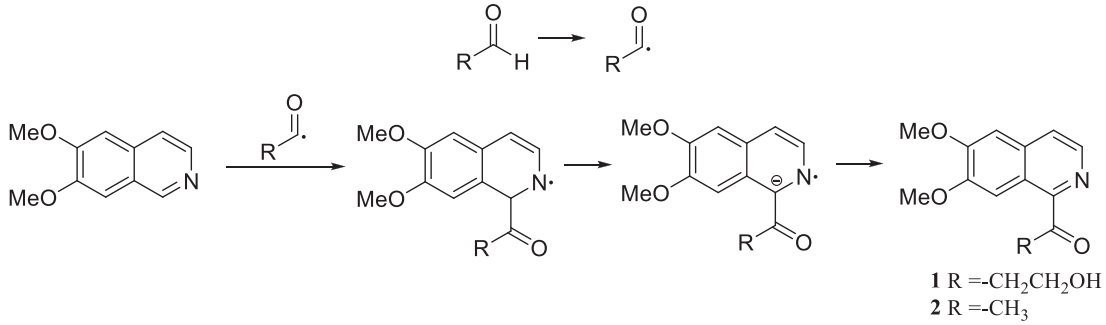
was supported by the HMBC correlation of the H_2-2′ (δ_H 3.24) with C-1 (δ_C 156.8). The HMBC correlations from two methoxy protons (δ_H 3.78 and 3.83) to C-6 (δ_C 152.3) and C-7 (δ_C 154.6) indicated that two methoxy groups was located at C-6, and C-7, respectively. Thus, the structure of **1** was established, and gave the trivail name of fistulatin A.

Fistulatin B (**2**) was obtained as a yellow amorphous powder and showed a quasi-molecular ion at *m/z* 254.0785 [M + Na]$^+$ in the HRESIMS (calcd *m/z* 254.0793), corresponding to the molecular formula $C_{13}H_{13}NO_3$. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**. The chemical shift differences resulted from the disappearance of a 3-hydroxypropanoyl resonance and appearance of an acetyl group resonance (δ_C 195.4 s and 26.8 q; δ_H 2.42) in **2**. This indicated that the 3-hydroxypropanoyl group at C-1 in **1** was converted into an acetyl group in **2**. The HMBC correlation of the acetyl proton resonance (δ_H 2.42) with C-1 (δ_C 155.3) indicated that the acetyl group was located at C-1. The HMBC correlations from two methoxy protons (δ_H 3.78 and 3.83) to C-6 (δ_C 152.1) and C-7 (δ_C 154.4) indicated that two methoxy group was located at C-6, and C-7, respectively. The structure of **2** was therefore defined.

Table 1. ^{1}H NMR and ^{13}C NMR Data (in C_5D_5N) of compounds **1** and **2**

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	156.8 s		155.3 s	
3	138.2 d	8.27 (d) 6.2	138.5 d	8.25 (d) 6.2
4	122.9 d	7.45 (d) 6.2	122.5 d	7.43 (d) 6.2
5	106.2 d	7.05 s	106.5 d	7.07 s
6	152.3 s		152.1 s	
7	154.6 s		154.4 s	
8	105.3 d	7.83 s	105.2 d	7.84 s
9	122.0 s		122.0 s	
10	136.8 s		136.5 s	
1′	197.9 s		195.4 s	
2′	39.8 t	3.24 (t) 6.8	26.8 q	2.42 s
3′	57.3 t	4.34 (t) 6.8		
-OMe-6	56.0 q	3.78 s	56.1 q	3.78 s
-OMe-7	56.2 q	3.83 s	56.8 q	3.83 s

Compounds **1** and **2** bear a 3-hydroxypropanoyl or an acetyl group at C-1, respectively. Referring to the literature,[22] we think that 3-hydroxypropanal and aldehyde form the radical in the first place, then addition of radical to the heteroarene provides the corresponding amidyl radical, after deprotonation and transfer a single electron giving the product (Scheme 1).



Scheme 1. The biogenesis of compounds **1** and **2**

Compounds **1**-**6** were tested for their anti-TMV activity. The anti-TMV activity was tested using the

half-leaf method. Ningnanmycin (a commercial product for plant disease in China) with inhibition rate of 30.8%, was used as a positive control.[23,24]

The antiviral inhibition rates of compounds **1-6** at the concentration of 20 μM were listed in Table 2. The results showed that compounds **1-6** exhibited weak anti-TMV activity with inhibition rates in the range of 15.4-23.5%.

Table 2. TMV Infection Inhibition Activity of Compounds **1–6**

Compound	Inhibition rates at 20 μM (%)	Compound	Inhibition rates at 20 μM (%)
1	23.5 ± 2.6	**5**	20.8 ± 2.5
2	21.3 ± 2.4	**6**	22.6 ± 2.8
3	18.8 ± 2.0	ningnanmycin	30.8 ± 3.2
4	15.4 ± 2.3		

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

EXPERIMENTAL

General. UV spectra were obtained using 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 DRX-500 spectrometers with TMS as internal standard, and the chemical shifts (δ) were expressed in ppm. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant material. The twigs of *C. fistula* were collected in Honghe prefecture of Yunnan Province, People's Republic of China, in September 2014. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-14-09-39) has been deposited in our Laboratory.

Extraction and Isolation. The air-dried and powdered twigs of *C. fistula* (2.6 kg) were extracted with 70% aq. acetone, and the extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9 with saturated Na_2CO_3 aq. and extracted with EtOAc. The EtOAc-soluble alkaloidal materials (15.6 g) were applied to silica gel (200–300 mesh) column chromatography, eluting with $CHCl_3$/MeOH gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A-F. Further separation of fraction C (9:1, 3.86 g) by silica gel column chromatography, eluted with $CHCl_3$/Me_2CO (9:1-2:1), yielded a mixture of C1–C7. Fraction C2 (8:2, 0.57 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (55% MeOH/H_2O, flow rate 12 mL/min) to give **4** (12.2 mg), **5** (13.6 mg), and **6** (15.8 mg). Fraction C3 (7:3, 0.82 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (47% MeOH/H_2O, flow rate 12 mL/min) to give **1** (12.2 mg) and **2** (15.9 mg). Fraction C4 (6:4, 0.62 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative

HPLC (42% MeOH/H_2O, flow rate 12 mL/min) to give **3** (16.4 mg).

Anti-TMV Assays. The anti-TMV activity was tested using the half-leaf method,[23,24] and ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as positive control.

Fistulalkaloid A (**1**): Obtained as yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 215 (4.28), 262 (3.58), 298 (3.13), 336 (3.42) nm; IR (KBr) ν_{max} 3418, 3126, 2953, 1657, 1622, 1568, 1455, 1372, 1236, 1150, 1046, 858 cm^{-1}; ^1H NMR and ^{13}C NMR data (in C_5D_5N, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 284 $[M + Na]^+$; HRESIMS *m/z* 284.0889 $[M + Na]^+$ (calcd for $C_{14}H_{15}NNaO_4$, 284.0899).

Fistulalkaloid B (**2**): Obtained as yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 218 (4.32), 260 (3.53), 295 (3.22), 330 (3.48) nm; IR (KBr) ν_{max} 3135, 2964, 1662, 1618, 1572, 1438, 1361, 1238, 1161, 1054, 846 cm^{-1}; ^1H NMR and ^{13}C NMR data (in C_5D_5N, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 254 $[M + Na]^+$; HRESIMS *m/z* 254.0785 $[M + Na]^+$ (calcd for $C_{13}H_{13}NNaO_3$, 254.0793).

ACKNOWLEDGEMENTS

This project was supported by the National Natural Science Foundation of China (No.21302164, 31460144, and 21562044) and the Applied Fundamental Foundation of Yunnan Province (No. 2013FB097).

REFERENCES (AND NOTES)

1. Delectis Flora Reipublicae Popularis Sinicae Edita, *Flora Reipublicae Popularis Sinicae*, Science Press, Beijing, 1988, **39**, 123.
2. Y. Hu, L. Q. Chen, X. Zhu, R. L. Wang, and L. Zhang, *Chin. Med. J. Res. Prac.*, 2013, **27**, 69.
3. Z. F. Xu, *Acta Bot. Yunnan*, 2008, **30**, 371.
4. Y.-K. Li, Y.-C. Yang, Y. Qin, Y. Meng, Y.-Q. Ye, H.-Y. Yang, X.-M. Gao, and Q.-F. Hu, *Heterocycles,* 2014, **89**, 481.
5. S. Aurapa and G. Wandee, *Int. J. Biomed. Pharm. Sci.*, 2009, **3**, 42.
6. P. Sartorelli, S. P. Andrade, M. S. Melhem, F. O. Prado, and A. G. Tempone, *Phytother. Res.*, 2007, **21**, 644.
7. M. M. Vaishnav, A. K. Tripathi, and K. R. Gupta, *Fitoterapia,* 1993, **64**, 93.
8. Y. K. Li, Y. L. Meng, Y. C. Yang, Y. Qin, C. F. Xia, Y. Q. Ye, X. M. Gao, and Q. F. Hu, *Phytochem. Lett.*, 2014, **10**, 46.
9. Y. H. Kuo, P. H. Lee, and Y. S. Wein, *J. Nat. Prod.*, 2002, **65**, 1165.
10. Q.-F. Hu, L.-M. Li, D.-L. Zhu, Z.-H. Yu, J.-B. Zhan, J. Lou, Y.-D. Wang, K. Zhou, M. Zhou, Y.-K. Li, and X.-M. Gao, *Heterocycles*, 2015, **91**, 1980.
11. W. Zhao, X. Y. Zeng, T. Zhang, L. Wang, G. Y. Yang, Y. K. Chen, Q. F. Hu, and M. M. Miao,

Phytochem. Lett., 2013, **6**, 179.

12. Q. F. Hu, D. Y. Niu, B. Zhou, Y. Q. Ye, G. Du, C. Y. Meng, and X. M. Gao, *Bull. Korean Chem. Soc.*, 2013, **34**, 3013.
13. X. M. Gao, Y. Q. Shen, X. Z. Huang, L. Y. Yang, L. D. Shu, Q. F. Hu, and G. P. Li, *J. Braz. Chem. Soc.*, 2013, **24**, 685.
14. M. Zhou, K. Zhou, X. M. Gao, Z. Y. Jiang, J. J. Lv, Z. H. Liu, G. Y. Yang, M. M. Miao, C. T. Che, and Q. F. Hu, *Org. Lett.*, 2015, **17**, 2638.
15. S. M. Kumar, A. Kumar, L. Kumar, B. K. Sarkhel, and M. J. Datta, *J. Freshwater Biol*., 1992, **4**, 17.
16. L. Q. Wang, Z. R. Tang, W. H. Mu, J. F. Kou, and D. Y. He, *J. Asian Nat. Prod. Res.*, 2013, **15**, 1210.
17. T. Kametani, S. Shibuya, and L. L. Lin, *Yakugaku Zasshi*, 1966, **86**, 973.
18. C. Y. Chen, F. R. Chang, W. B. Pan, and Y. C. Wu, *Phytochemistry*, 2001, **56**, 753.
19. Y. W. Tao, X. J. Zeng, C. B. Mou, J. Li, X. L. Cai, Z. G. She, S. N. Zhou, and Y. C. Lin, *Magn. Reson. Chem*., 2008, **46**, 501.
20. M. H. Abu Zarga, G. A. Miana, and M. Shamma, *Heterocycles*, 1982, **18**, 63.
21. Q. F. Hu, B. Zhou, X. M. Gao, L. Y. Yang, L. D. Shu, Y. Q. Shen, G. P. Li, C. T. Che, and G. Y. Yang, *J. Nat. Prod*., 2012, **75**, 1909.
22. S. Yogesh, L. Manjunath, and R. P. Kandikere, *J. Org. Chem.*, 2014, **79**, 3856.
23. Q. F. Hu, B. Zhou, J. M. Huang, X. M. Gao, L. D. Shu, G. Y. Yang, and C. T. Che, *J. Nat. Prod*., 2013, **76**, 292.
24. M. Zhou, M. M. Miao, G. Du, S. Z. Shang, W. Zhao, Z. H. Liu, G. Y. Yang, C. T. Che, Q. F. Hu, and X. M. Gao. *Org. Lett*., 2014, **16**, 5016.