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NOTE

A New Anthraquinone from the Root of *Cassia fistula* and Their Antitobacco Mosaic Virus Activity

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A new anthraquinone, fistulaquinone 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 anti-tobacco mosaic virus activity. The compound shows modest anti-tobacco mosaic virus activity with inhibition rates of 16.3 %.

Key Words: Anthraquinone, *Cassia fistula*, Antitobacco mosaic virus activity.

The plant of *Cassia fistula* L., (Leguminosae) 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]. Previous phytochemical studies of *C. fistula* have shown the presence of anthraquinones[3], steroids[4], chromones[5,6] and flavonoids[7]. Continuing the efforts to discover bioactive metabolites from local plants, we now investigated the chemical constituents of the root of *C. fistula*, leading to the isolation of a new anthraquinone (**1**) (Fig. 1). The structures of the isolated compounds were determined by means of spectroscopic methods including 1D and 2D NMR techniques and compounds **1** exhibited modest *in vitro* antitobacco mosaic virus activity.

UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. 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 Dehong Prefecture, Yunnan Province, P.R. China, in September 2011. The identification of the plant material was verified by Dr. N. Yuan of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (YNNU 11-9-15) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered roots of *C. fistula*. (5.2 kg) were extracted four times with 70 % methanol (4 L × 2.0 L) at room temperature and filtered. The crude extract (326 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 (35 % methanol, flow rate 12 mL/min) to give anthraquinone **1** (25.8 mg).

Fistulaquinone A: $C_{22}H_{22}O_5$, obtained as yellow powder; UV (MeOH), λ_{max} (log ε) 278 (4.15), 257 (3.96), 215 (4.34) nm; IR (KBr, ν_{max}, cm^{-1}): 3386, 2924, 2878, 1698, 1639, 1605, 1572, 1496, 1418, 1362, 1273, 1157, 1126, 1060, 887, 768; ^{1}H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), Table-1; ESI-MS (positive ion mode) *m/z* 389 $[M + Na]^+$; HR-ESI-MS (positive ion mode) *m/z* 389.1371 $[M + Na]^+$ (calcd. (%) 389.1365 for $C_{22}H_{22}NaO_5$).

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.

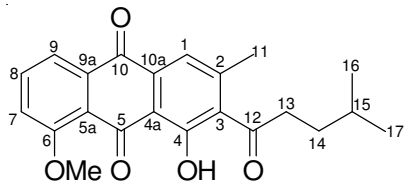

Fig. 1. Structure of new anthraquinone

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

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	122.8 d	7.64, s	9a	133.9 s	–
2	144.9 s	–	10	178.9 s	–
3	136.2 s	–	10a	122.6 s	–
4	159.5 s	–	11	19.6 q	2.39, s
4a	114.8 s	–	12	205.2 s	–
5	188.7 s	–	13	41.3 t	2.85, t, *J* = 7.1
5a	114.2 s	–	14	31.8 t	1.57, m
6	164.8 s	–	15	27.4 d	1.68, m
7	124.0 d	7.35, d, *J*=8.4	16,17	21.2 q	0.96, d, *J* = 6.2
8	137.8 d	7.71, dd, *J* = 8.4, 7.5	-OMe-6	55.8 q	3.82, s
9	120.0 d	7.80, d, *J* = 7.5	Ar-OH-4	–	12.05, brs

Compound **1** was isolated as a yellow powder. High-resolution ESIMS analysis gave an quasi-molecular ion at *m/z* 389.1371 $[M + Na]^+$, consistent with a molecular formula of $C_{22}H_{22}O_5$, which indicated 12 degrees of unsaturation. The UV spectrum of **1** exhibited absorption bands at 278, 257 and 215 nm, highly suggesting an anthraquinone chromophore[8]. Strong absorption bands accounting for hydroxy (3386 cm^{-1}), carbonyl (1698, 1639) and aromatic groups (1605, 1572, 1496, 1418 cm^{-1}) could also be observed in its IR spectrum. The ^{1}H NMR spectrum of **1** (Table-1) showed the presence of one phenolic proton at δ_H 12.05, one singlet aromatic proton at δ_H 7.64 (s, H-1), three aromatic protons at δ_H 7.35 (d, *J* = 8.4 Hz, H-7), 7.71 (dd, *J* = 8.4, 7.5 Hz, H-8) and 7.80 (d, *J* = 7.5 Hz, H-9) and 17 aliphatic protons contributed by one methyl singlet at δ_H 2.39 (H-11), two methyl doublets at δ_H 0.96 (H-16 and H-17), two methylenes at δ_H 2.85 and 1.57 (H-13 and H-14, respectively), one methine at δ_H 1.68 (H-15) and one O-methyl singlet at δ_H 3.82 (-OMe-6). In the ^{13}C NMR spectrum of **1** (Table-1), 14 sp2 carbon signals, including two oxygenated quaternary sp2 carbon signals at δ_C 159.5 and 164.8 and two carbonyl carbon signals at δ_C 178.9 and 188.7 were observed, which highly suggested the presence of anthraquinone core[8]. An additional carbonyl at δ_C 205.2 and seven aliphatic carbons (δ_C 19.6, 21.2, 21.2, 27.4, 31.8, 41.3 and 55.8) account for the remaining substituents on the anthraquinone ring.

The substituents and their location on the anthraquinone ring were established by analysis of the COSY and HMBC spectra of **1** (Fig. 2). COSY correlations between H-7/H-8 and H-8/H-9, combined with HMBC correlations from H-9 (δ_H 7.80) to C-7 (δ_C 124.0), C-5a (δ_C 114.2) and C-10 (δ_C 178.9), from H-8 (δ_H 7.71) to C-6 (δ_C 164.8), C-9a (δ_C 133.9) and C-9 (δ_C 120.0) and from H-7 (δ_H 7.35) to C-5a (δ_C 114.2) and C-9 (δ_C 120.0), as well as from the O-methyl proton (δ_H 3.82) to C-6 (δ_C 164.8) established the 1,2,3-trisubstitued benzene ring with a O-methyl at C-6. The presence of a 4-methylpentyl side chain was suggested by COSY correlations (Fig. 2) and supported by HMBC correlations from H-13 (δ_H 2.85) to C-12 (δ_C 205.2), C-14 (δ_C 31.8) and C-15 (δ_C 27.4) and from H-16, 17 (δ_H 0.96) to C-14 (δ_C 31.8), C-15 (δ_C 27.4). Additional HMBC correlations from a methyl singlet at δ_H 2.39 (H-11) to C-1 (δ_C 122.8), C-2 (δ_C 144.9) and C-3 (δ_C 136.2) and from H-1 (δ_H 7.64) to C-3 (δ_C 136.2), C-10 (δ_C 178.9), C-11 (δ_C 19.6) and C-4a (δ_C 114.8), as well as from the phenolic proton at δ_H 12.05 (OH-4) to C-3 (δ_C 136.2), C-4a (δ_C 114.8) and C-4 (δ_C 159.2) established the location of a methyl at C-2 and a hydroxy group at C-4, which in turn indicated the 4-methylpentyl side chain was located at C-3 of the anthraquinone ring. As a result, the full structure of **1** was established as 2-(4-methylpentanoyl)-1-hydroxy- 8-methoxy-3-methylanthracene-9,10-dione and gives the trivial name of fistulaquinone A.

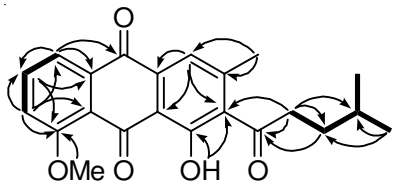

Fig. 2. COSY (–) and key HMBC (↷) correlations of **1**

Since some of the phenolic compounds exhibited anti virus activities[9,10], compounds **1** was tested for it antitobacco mosaic virus activity. The anti TMV activities were tested using the half-leaf method[11]. 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 16.3 %.

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REFERENCES

1. V. Duraipandiyan and S. Ignacimuthu, *J. Ethnopharmacol*., **112**, 590 (2007).
2. J. Ma, L.X. Zhang and Y.H. Guan, *Chin. J. Ethnomed. Ethnopharm*., **5**, 178 (2004).
3. S. Aurapa and G. Wandee, *Int. J. Biomed. Pharm. Sci*., **3**, 42 (2009).
4. P. Sartorelli, S.P. Andrade, M.S. Melhem, F.O. Prado and A.G. Tempone, *Phytother. Res*., **21**, 644 (2007).
5. Z. Zuraini, C. Yeng, L.L. Yee, Y.L. Lachimanan, N.S. Lai and S. Sreenivasan, *Molecules,* **16**, 7583 (2011).
6. 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*., **75**, 1909 (2012).
7. X.M. Gao, L.D. Shu, L.Y. Yang, Y.Q. Shen, M.Z. Cui, X.M. Li and Q.F. Hu, *Heterocycles*, **87**, 125 (2013).
8. Y.C. Hu, E.D. Martinez and J.B. MacMillan, *J. Nat. Prod*., **75**, 1759 (2012).
9. X.M. Gao, L.Y. Liying Yang, Y.Q. Shen, L.D. Shu, X.M. Li and Q.F. Hu, *Bull. Korean. Chem. Soc*., **33**, 2447 (2012).
10. X.M. Gao, X.S. Li, X.Z. Yang, H.X. Mu, Y.K. Chen, G.Y. Yang and Q.F. Hu, *Heterocycles*, **85**, 147 (2012).
11. 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).