Neolignans from Flower Buds of *Magnolia fargesii* and their Antitobacco Mosaic Virus Activities

YE-KUN YANG^{1,2}, HAI-TAO HUANG², JIAN-HUA CHEN², QIU-FEN HU³, XUE-MEI LI^{2*}, JUN ZHOU¹ and ZHONG-TAO DING^{1,*}

¹School of Chemical Science and Technology, Yunnan University, Kunming 650091, P.R. China

²Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P.R. China

³Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities, Kunming 650031, P.R. China

*Corresponding authors: E-mail: xmli@cyats.com; huqiufena@aliyun.com

(Received: 4 January 2013;

Accepted: 7 October 2013)

AJC-14238

A new neolignan, fargesilignan A (**1**), together with five known neolignans (**2-6**), were isolated from the flower buds of *Magnolia fargesii*. The structures of **1-6** were elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compounds **1-6** were tested for their antitobacco mosaic virus activities. Compounds **2** and **5** (20 μ M) showed moderate antitobacco mosaic virus activity with inhibition rates of 14.5 and 22.2 %.

Key Words: *Magnolia fargesii*, Neolignans, Antitobacco mosaic virus activities.

INTRODUCTION

The dried flower buds of *Magnolia fargesii*, with trivial name of Xinyi, has been used as herb medicine for the treatment of inflammatory-related diseases such as nasal congestion, empyema, sinusitis and allergic rhinitis^{1,2}. Previous phytochemical investigations have reported that this species contains several secondary metabolites such as lignans³⁻⁵, neolignans^{6,7}, sesquiterpenes^{2,8} and essential oils⁹, which show various biological activities.

To search for more new bioactive compounds from this plant, we reexamined the flower buds of *M. fargesii*, which led to the isolation of a new neolignan (**1**) and five known neolignans (**2-6**). In addition, the antitobacco mosaic virus activities were evaluated. Their structure elucidation and biological activities are described in this paper.

EXPERIMENTAL

UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectro-photometer 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. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. 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 $\times$ 25 cm, 7 μ m) column or a Venusil MP C₁₈ (20 mm $\times$ 25 cm, 5 μ m) column. Column chromatography was performed with Si gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, 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 Si gel plates sprayed with 5 % sulfuric acid in ethanol.

The flower buds of *M. fargesii*, indigenous to Leshan country, Sicuan province, were purchased from Kunming Herb Medicine Market in September 2011. A voucher specimen (YU-11-9-21) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered flower buds of *Magnolia fargesii* (4.0 kg) were extracted four times with 70 % aqueous acetone (4 L $\times$ 3) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (4 L $\times$ 3). The EtOAc partition (185 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a CHCl₃-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. Further separation of fraction B (28.3 g) by silica gel column chromatography, eluted with CHCl₃-acetone (9:1-2:1), yielded mixtures B1-B6. Fraction B1 (9:1, 5.5 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (65 % MeOH-H₂O, flow rate 12 mL/min) to give **4** (18.9 mg) and **5** (23.2 mg). Fraction B3 (8:2, 6.22 g) was subjected to silica gel column chromatography using

CHCl_3 -acetone and semi-preparative HPLC (52 % MeOH - H_2O , flow rate 12 mL/min) to give **1** (22.3 mg), **2** (24.5 mg) and **6** (17.4 mg). Fraction B3 (7:3, 1.74 g) was subjected to silica gel column chromatography using CHCl_3 -acetone and semi-preparative HPLC (40 % MeOH - H_2O , flow rate 12 mL/min) to give **3** (18.5 mg).

Antitobacco mosaic virus assays: The antitobacco mosaic virus activities were tested using the half-leaf method²⁰. Ningnanmycin (2 % water solution), a commercial product for plant disease in China, was used as a positive control.

Fargesilignan A (1): White powder; UV (MeOH), λ_{max} (log ϵ) 352 (4.02), 308 (3.65), 256 (3.72), 210 (4.48) nm; IR (KBr, ν_{max} , cm^{-1}): 3408, 2972, 2893, 1682, 1622, 1586, 1455, 1316, 1205, 1105; ESIMS (positive ion mode) m/z 365 [$\text{M} + \text{Na}$]⁺; HRESIMS (positive ion mode) m/z 365.1008 [$\text{M} + \text{Na}$]⁺ (calcd. (%) 365.1001 for $\text{C}_{19}\text{H}_{18}\text{NaO}_6$).

RESULTS AND DISCUSSION

A 70 % aqueous acetone extract prepared from the flower buds of *M. fargesii* was partitioned between EtOAc and H_2O . The EtOAc layer was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1-6**, including three new neolignans named fargesilignan A (**1**), together with six known neolignans,

biondinin A (**2**)¹⁰, clinopodphenol A (**3**)¹¹, fargesiiphenol A (**4**)¹², morinol G (**5**)¹³, feddeiphenol A (**6**)¹⁴. The structures of compounds **1-6** are shown in Fig. 1 and the ^1H and ^{13}C NMR data of compound **1** are listed in Table-1.

Compound **1** was isolated in the form of a white powder, with a melting point of 202–204 °C. The high resolution mass spectrum utilizing the ESI-ionization mode showed the peak of the molecule at m/z 365.1008 [$\text{M} + \text{Na}$]⁺, compatible with the molecular formula $\text{C}_{19}\text{H}_{18}\text{O}_6$ [calcd. (%) 365.1001]. The IR spectrum revealed the presence of a wide band between 3408 cm^{-1} and a band at 1682 cm^{-1} , characteristic of O-H and C=O, respectively. Absorption was also observed at 1624–1458 cm^{-1} , characteristic of C=C stretching of an aromatic ring and between 1316–1105 cm^{-1} of C-O stretching. The ^{13}C and DEPT NMR spectrum showed the presence of 19 signals (Table-1), corresponding to two aromatic rings (C-1-C-6 and C-1'-C-6'), a pair of double bond (C-7' and C-8'), a 3-hydroxypropan-1-one moiety (C-7-C-9)¹⁵, two methoxy groups (δ_{C} 55.8, 55.9; δ_{H} 3.82, 3.86) and a phenolic hydroxy group (δ_{H} 11.22). Based on comparison with ^{13}C NMR spectral data of **1** with these of the known compound, sobraline¹⁶, the major differences due to a carboxyl in sobraline replaced by a 3-hydroxypropan-1-one moiety in **1**. The ^1H NMR spectrum of this compound indicated the presence of a signal at δ_{H} 7.08 (s) characteristic

Fig. 1. Structures of neolignans from *M. fargesii*

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

No.	δ_{C} (m)	δ_{H} (m, J, Hz)	No.	δ_{C} (m)	δ_{H} (m, J, Hz)
1	125.2 s	—	2'	157.4 s	—
2	121.8 d	8.11, d, $J = 1.8$	3'	96.8 d	6.34, d, $J = 2.5$
3	131.8 s	—	4'	162.5 s	—
4	124.1 d	7.92, dd, $J = 1.8, 8.5$	5'	92.9 d	6.25, d, $J = 2.5$
5	111.8 d	7.62, dd, $J = 8.5$	6'	160.5 d	—
6	157.5 s	—	7'	153.9 s	—
7	198.2 s	—	8'	108.4 d	7.08, s
8	43.8 t	3.20, t, $J = 6.2$	-OMe-4'	55.8 q	3.86, s
9	61.8 t	4.33, t, $J = 6.2$	-OMe-6'	55.9 q	3.82, s
1'	102.6 s	—	Ar-OH-2'	—	11.22, s

of H-8', as well as signals at δ_{H} 8.11 (d, $J = 1.8$ Hz), 7.92 (dd, $J = 1.8$ and 8.5 Hz) and 7.62 (d, $J = 8.5$ Hz), assigned to the hydrogens H-2, H-4 and H-5, respectively and signals at δ_{H} 6.25 (d, $J = 2.5$) and 6.24 (d, $J = 2.5$), corresponding to the hydrogens H-3' and H-5', respectively. It was also observed two singlets at δ_{H} 3.82 and 3.86, the first referring to the methoxyl at C-4' and the second to the methoxyl at C-6' and a broad singlet at δ_{H} 11.22 for phenolic hydroxyl at C-2'. Finally, two triplets at δ_{H} 3.20 and δ_{H} 4.33 were assigned to C-8 and C-9. These assignments were confirmed by the direct correlations observed in the HMQC spectrum. The HMBC spectrum showed correlations between the hydrogens at δ_{H} 8.11 (H-2) and 7.92 (H-4) with the signal at δ_{C} 198.2 (C-7) and δ_{H} 3.20 (H-8) with δ_{C} 131.8 (C-3) assigned to the 3-hydroxypropan-1-one moiety, confirming its insertion at C-3. Two methoxy groups at C-4' and C-6' and a phenolic hydroxy group at C-2' were also confirmed by the HMBC correlations (Fig. 2). Therefore, the structure of compound **1** was assigned as 3-hydroxy-1-(2-(2-hydroxy-4,6-dimethoxyphenyl) benzofuran-5-yl) propan-1-one and gives the trivial name of fargesilignan A.

Fig. 2. Selected HMBC (→) correlations of compound **1**

Many polyphenols are known to exhibit antitobacco mosaic virus activities¹⁷⁻¹⁹. Compounds **1-6** were therefore tested for antitobacco mosaic virus activities using the half-leaf method²⁰. Ningnanmycin, a biochemical pesticide against virus diseases on tobacco, with inhibitory of 31.8 %, was used as the positive control. Compounds **2** and **5** (20 μM) showed moderate antitobacco mosaic virus activity with inhibition rates of 14.5 and 22.2 %. Compounds **1**, **3**, **4** and **6** showed low antitobacco mosaic virus activities with inhibitory rates below 10 %.

ACKNOWLEDGEMENTS

This project was supported financially by the Excellent Scientific and Technological Team of Yunnan High School (2010CI08) and Green Chemistry and Functional Materials Research for Yunnan Innovation Team (2011HC008).

REFERENCES

1. M. Miyazawa, H. Kasahara and H. Kameoka, *Phytochemistry*, **31**, 3666 (1992).
2. Y.J. Lee, Y.M. Lee, C.K. Lee, J.K. Jung, S.B. Han and J.T. Hong, *Pharm. Therap.*, **130**, 157 (2011).
3. Y.L. Huang, C.C. Chen, Y.P. Chen, H.Y. Hsu and Y.H. Kuo, *Planta Med.*, **56**, 237 (1990).
4. M. Miyazawa, Y. Ishikawa, H. Kasahara, J. Yamanaka and H. Kameoka, *Phytochemistry*, **35**, 611 (1994).
5. J. Lee, D. Lee, D.S. Jang, J.W. Nam, J.P. Kim, K.H. Park, M.S. Yang, E.K. Seo, *Chem. Pharm. Bull. (Tokyo)*, **55**, 137 (2007).
6. J. Lee, E.K. Seo, D.S. Jang, T.J. Ha, J.P. Kim, J.W. Nam, G. Bae and J.S. Kim, *Chem. Pharm. Bull.*, **57**, 298 (2009).
7. X.M. Gao, Y.Q. Shen, L.Y. Yang, L.D. Shu, G.P. Li and Q.F. Hu, *J. Braz. Chem. Soc.*, **23**, 1274 (2012).
8. K.Y. Jung, D.S. Kim, S.H. Park, I.S. Lee, S.R. Oh, J.J. Lee, E.H. Kim and H.K. Lee, *Phytochemistry*, **48**, 1383 (1998).
9. J.Y. Fang, T.H. Tsai, C.F. Hung and W.W. Wong, *J. Pharm. Pharmacol.*, **56**, 1493 (2004).
10. Y.L. Ma, Q. Huang and G.Q. Han, *Phytochemistry*, **41**, 287 (1996).
11. Q.F. Hu, H.X. Mu, H.T. Huang, H.Y. Lv, S.L. Li and G.Y. Yang, *Chem. Nat. Compd.*, **48**, 198 (2012).
12. J. Lee, E.K. Eo, D.S. Jang, M.S. Yang and J.S. Kim, *Chem. Pharm. Bull.*, **57**, 298 (2009).
13. B.N. Su, Y. Takaishi and T. Kusumi, *Tetrahedron*, **55**, 14571 (1999).
14. Q.F. Hu, H.X. Mu, H.T. Huang, H.Y. Lv, S.L. Li, G.Y. Yang, P.F. Tu and G.P. Li, *Chem. Pharm. Bull. (Tokyo)*, **59**, 1421 (2012).
15. 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).
16. S.A.L. Madeiro, H.F.S. de Lucena, C.D. Siqueira, M.C. Duarte, R. Braz-Filho, J.M.B. Filho, M.S. da Silva and J.F. Tavares, *J. Braz. Chem. Soc.*, **23**, 2322 (2012).
17. 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).
18. Z.R. Gao, S.T. Xu, J. Wei, H.L. Shi, Z. Li and Q.F. Hu, *Asian J. Chem.*, **25**, 2747 (2013).
19. 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).
20. B.A. Song, H.P. Zhang, H. Wang, S. Yang, L.H. Jin, D.Y. Hu, L.L. Pang and W. Xue, *J. Agric. Food Chem.*, **53**, 7886 (2005).