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

YE-KUN YANG[1,2], HAI-TAO HUANG[2], JIAN-HUA CHEN[2], QIU-FEN HU[3], XUE-MEI LI[2,*], JUN ZHOU[1] and ZHONG-TAO DING[1,*]

[1]School of Chemical Science and Technology, Yunnan University, Kunming 650091, 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 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

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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 μM) 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 lignans[3-5], neolignans[6,7], sesquiterpenes[2,8] and essential oils[9], 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 neolignan (**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 × 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), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany) and MCI gel (75-150 μm, 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 × 3) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (4 L × 3). The EtOAc partition (185 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a $CHCl_3$-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 $CHCl_3$-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-H_2O, 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 method[20]. 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 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 365.1008 $[M + Na]^+$ (calcd. (%) 365.1001 for $C_{19}H_{18}NaO_6$).

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 ºC. The high resolution mass spectrum utilizing the ESI-ionization mode showed the peak of the molecule at *m/z* 365.1008 $[M + Na]^+$, compatible with the molecular formula $C_{19}H_{18}O_6$ [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, sobraline[16], 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

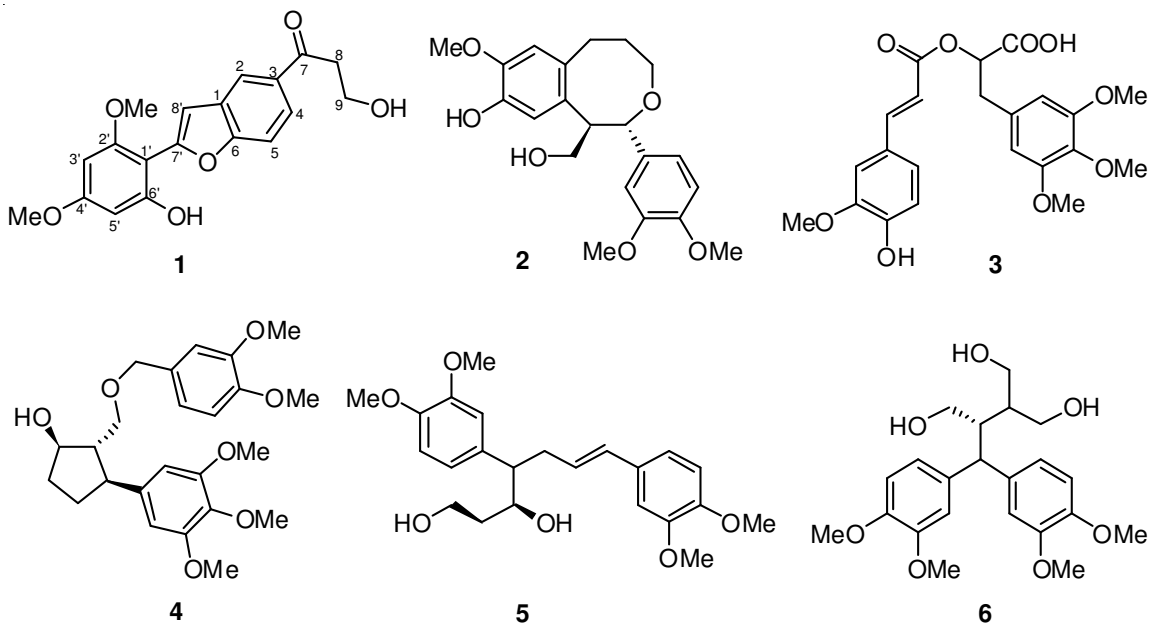

Fig. 1. Structures of neolignans from *M. fargesii*

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-hydroxy-propan-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.

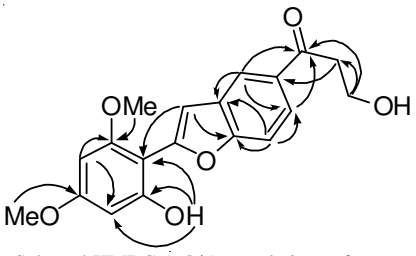

Fig. 2. Selected HMBC ( ) correlations of compound **1**

Many polyphenols are known to exhibit antitobacco mosaic virus activities[17-19]. Compounds **1-6** were therefore tested for antitobacco mosaic virus activities using the half-leaf method[20]. 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 anti-tobacco 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 %.

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