8-O-4’-Neolignans from Flower Buds of *Magnolia fargesii* and their Biological Activities

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Três novos derivados de 8-O-4’-neolignanas, fargesifenols A-C, juntamente com seis neolignanas conhecidas, foram isoladas de botões de flores de *Magnolia fargesii*. As estruturas foram elucidadas por métodos espectroscópicos, incluindo técnicas de RMN 1D e 2D. Os compostos foram também testados quanto à sua atividade anti-HIV-1 e quanto às citotoxicidades.

Three new 8-O-4’-neolignans, fargesifenols A-C, together with six known neolignans, were isolated from the flower buds of *Magnolia fargesii*. The structures were elucidated by spectroscopic methods, including extensive 1D and 2D-NMR techniques. Compounds were also tested for their anti-HIV-1 activities and cytotoxicities.

**Keywords:** *Magnolia fargesii*, 8-O-4’-neolignans, anti-HIV-1 activity, cytotoxicity

**Introduction**

The genus *Magnolia* (Magnoliaceae) has traditionally been used as herb medicine in China for a long time. Especially, Xinyi (dried flower buds of *Magnolia fargesii*), has been used for the treatment of inflammatory-related diseases such as nasal congestion, empyema, sinusitis, and allergic rhinitis. Previous phytochemical investigations have reported that this species contains several secondary metabolites such as lignans, neolignans, sesquiterpenes, and essential oils, 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 three new 8-O-4’-neolignans, named fargesifenols A-C (1-3), along with six known compounds (4-9). In addition, the anti-HIV-1 activities and their cytotoxicities were evaluated. Their structure elucidation and biological activities are described in this paper.

**Results and Discussion**

A 70% aq. acetone extract prepared from the flower buds of *M. fargesii* was partitioned between EtOAc and H$_2$O. The EtOAc layer was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds 1-9, including three new 8-O-4’-neolignans named fargesifenols A-C (1-3), together with six known neolignans, polysyphorin (4), violin (5), 75,8S-threo-4,7,9,9’-tetrahydroxy-3,3’-dimethoxy-8-O-4’-neolignan (6), 75,8R-erythro-4,7,9,9’-tetrahydroxy-3,3’-dimethoxy-8-O-4’-neolignan (7), (7R,8S)-1-(3,4-dimethoxyphenyl)-2-[4-(3-hydroxy-1-propenyl)-methoxy-phenoxyl]-propane-1,3-diol (8), and rhaphidecursinol A (9). The structures of compounds 1-9 are shown in Figure 1, and the 1H and 13C NMR data of compounds 1-3 are listed in Table 1.

Compound 1 was obtained as pale yellow gum. Its molecular formula was determined as C$_{21}$H$_{26}$O$_{10}$ from the HRESIMS quasi-molecular ion peak [M+Na]$^+$ at m/z 397.1622 (calc. 397.1627). Its 1H and 13C NMR spectra showed signals of 26 hydrogens and 21 carbons, respectively, corresponding to two aromatic rings with five aromatic protons ($\delta_H$ 6.99, 7.34, 7.09, 7.15, 6.98), two methyl groups ($\delta_C$ 14.5, 18.4), three methoxy groups ($\delta_C$ 55.9, 55.9, 60.4), two oxidated methine groups ($\delta_C$ 75.1, 80.9), one allyl group ($\delta_C$ 131.4, 124.1, 18.4; $\delta_H$ 6.38 d J 15.8, 6.10-6.17 m, 1.75 d J 6.5), and a phenolic hydroxyl group ($\delta_H$ 11.25). Strong absorption bands accounting for hydroxyl (3498 cm$^{-1}$) and aromatic groups

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(1608, 1520, 1478 cm\(^{-1}\)) could also be observed in its IR spectrum. The UV spectrum of 1 showed absorption maxima at 285 and 210 nm, which confirmed the existence of the aromatic functions. The \(^1H\)–\(^1H\) COSY correlations \(\text{H-7}/\text{H-8}/\text{H-9}, \text{H-7’}/\text{H-8’}/\text{H-9’}\), together with HMBC correlations (Figure 2) of \(\text{H-7} (\delta_H 5.38)\) with \(\text{C-8} (\delta_C 80.9)\), \(\text{C-9} (\delta_C 14.5)\), \(\text{C-1} (\delta_C 139.5)\), \(\text{C-2} (\delta_C 102.9)\) and \(\text{C-6} (\delta_C 109.0)\), of \(\text{H-7’} (\delta_H 6.38)\) with \(\text{C-2’} (\delta_C 110.7)\), \(\text{C-1’} (\delta_C 132.6)\) and \(\text{C-6’} (\delta_C 119.3)\), and \(\text{H-8} (\delta_H 4.88)\) with \(\text{C-1} (\delta_C 139.5)\) and \(\text{C-4’} (\delta_C 147.4)\), suggested that 1 is an 8-\(O\)-4’-neolignan possessing three methoxyl groups and a phenolic hydroxyl group.

Three methoxyl groups located at C-3, C-4, C-3’ were assigned from the HMBC correlations of the methoxyl proton signals (\(\delta_H 3.77, 3.88, 3.74\)) with C-3 (\(\delta_C 153.9)\), C-4 (\(\delta_C 136.7)\), and C-3’ (\(\delta_C 151.9)\), respectively. The presence of a phenolic hydroxyl group at C-5 was supported by the HMBC correlations of the phenolic hydroxyl proton signal (\(\delta_H 11.25\)) with C-4 (\(\delta_C 136.7)\), C-5 (\(\delta_C 151.9)\), and C-6 (\(\delta_C 109.0)\), respectively. Thus, the plain structure of 1 was established.

The configuration of the two chiral carbons (C-7 and C-8) was considered to be threo according to the coupling constant (\(J 7.2 \text{ Hz}\)) between H-7 and H-8,\(^{15}\) which is distinct from that of the erythro diastereoisomers.\(^{16,17}\) The absolute configurations at C-7 and C-8 of 1 could be established on the basis of ROESY and CD spectroscopic evidence.\(^{18}\)

The CD spectrum of 1 gave a positive cotton effect at 237 nm, and clear ROESY correlations observed between H-7 and H-8, H-7 and H-2, H-7 and H-3–9 (Figure 3) indicated the 7S,8S-configuration of 1, which was named fargesiphenol A.

Compounds 2 and 3 (fargesiphenols B and C) were all obtained as yellow gums. HRESIMS analysis of 2 demonstrated that it has the molecular formula C\(_{22}\)H\(_{28}\)O\(_7\) (m/z 427.1730, for [M+Na]\(^+\), calc. 427.1733). The NMR spectra of 2, when compared to those of 1, displayed an additional methoxy group (\(\delta_H 3.85\); \(\delta_C 55.6\)), which was located at C-5’ from the HMBC cross-peak between this carbon (\(\delta_C 152.3)\) and the methoxyl protons (\(\delta_C 3.85)\).

The NMR spectra of 3, when compared to those of 2, displayed the characteristic signals of an acetoxy group (\(\delta_H 2.19\), \(\delta_C 21.1\) and \(\delta_C 169.9)\). Its \(^{13}\)C NMR spectrum
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showed downfield-shifted signals for C-4 ($\delta_c$ 142.1) and C-6 ($\delta_c$ 111.4), and an upfield-shifted signal for C-5 ($\delta_c$ 144.8), thus corroborating the placement of the acetoxyl group at C-5. According to the observed ROESY correlations and comparison of $^1$H and $^{13}$C NMR data with those of 1 (Table 1), the relative configurations of 2 and 3 were established as being the same as that of 1. The configuration of the two chiral carbons (C-7 and C-8) for 2 and 3 was considered to be $\text{threo}$ according to the coupling constant ($J = 7.2 \text{ Hz}$) between H-7 and H-8, and a positive Cotton effect at 237 nm for 2, and 238 nm for 3 in their CD spectrum indicated a $7^S, 8^S$-configuration.

Since some neolignans are reported to possess anti-HIV activities, these have been tested for compounds 1-9. The cytotoxicity assay against C8166 cells (CC$_{50}$), and anti-HIV-1 activity were evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC$_{50}$), using azidothymidine (AZT) as a positive control (EC$_{50}$ = 0.034 µg mL$^{-1}$ and CC$_{50}$ = 200 µg mL$^{-1}$). The results (Table 2) revealed significant activity for compounds 2, 6, 7, and 8, with therapeutic index (TI) values above 30. Compounds 1, 4, and 9 showed moderate activity with TI values above 10.

Some neolignans are also reported to possess cytotoxic activities, which led us to evaluate compounds 1-9 for their cytotoxicities. The cytotoxicity tests were performed in triplicate using a previously reported procedure. In the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the IC$_{50}$ was defined as the concentration of the tested compound resulting in a 50%
reduction of absorbance compared with untreated cells. The cytotoxic activities against HL-60, Hep-G2, KB, and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control) are shown in Table 3. Compound 2 revealed high cytotoxic activity to HL-60 and MDA-MB-231 cells, whereas compound 8 showed high cytotoxic activity to KB cells, both with IC_{50} values close to those of positive control. The other compounds displayed moderate or weak cytotoxic activity.

Table 2. Anti-HIV activities of the compounds 1-9

<table>
<thead>
<tr>
<th>Compound</th>
<th>CC_{50}/(μg mL^{-1})</th>
<th>EC_{50}/(μg mL^{-1})</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63.5</td>
<td>3.62</td>
<td>17.5</td>
</tr>
<tr>
<td>2</td>
<td>≥ 200</td>
<td>4.86</td>
<td>≥ 41.2</td>
</tr>
<tr>
<td>3</td>
<td>28.6</td>
<td>3.11</td>
<td>9.20</td>
</tr>
<tr>
<td>4</td>
<td>22.6</td>
<td>2.16</td>
<td>10.5</td>
</tr>
<tr>
<td>5</td>
<td>61.5</td>
<td>6.87</td>
<td>8.95</td>
</tr>
<tr>
<td>6</td>
<td>135.6</td>
<td>3.87</td>
<td>35.0</td>
</tr>
<tr>
<td>7</td>
<td>185.4</td>
<td>5.54</td>
<td>33.5</td>
</tr>
<tr>
<td>8</td>
<td>≥ 200</td>
<td>2.08</td>
<td>≥ 96.2</td>
</tr>
<tr>
<td>9</td>
<td>87.9</td>
<td>3.09</td>
<td>28.4</td>
</tr>
</tbody>
</table>

\[T_L = \frac{E_{C_{50}}}{CC_{50}}\]

Table 3. Cytotoxicities of compounds 1-9

<table>
<thead>
<tr>
<th>Compounds</th>
<th>HL-60</th>
<th>HepG2</th>
<th>KB</th>
<th>MDA-MB-231</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC_{50}/(μmol L^{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12.0</td>
<td>6.08</td>
<td>9.21</td>
<td>6.48</td>
</tr>
<tr>
<td>2</td>
<td>1.69</td>
<td>3.50</td>
<td>2.05</td>
<td>2.19</td>
</tr>
<tr>
<td>3</td>
<td>10.3</td>
<td>6.50</td>
<td>8.55</td>
<td>7.19</td>
</tr>
<tr>
<td>4</td>
<td>5.28</td>
<td>11.4</td>
<td>14.4</td>
<td>6.25</td>
</tr>
<tr>
<td>5</td>
<td>7.01</td>
<td>6.42</td>
<td>15.7</td>
<td>6.15</td>
</tr>
<tr>
<td>6</td>
<td>11.0</td>
<td>8.58</td>
<td>9.59</td>
<td>8.23</td>
</tr>
<tr>
<td>7</td>
<td>8.17</td>
<td>6.29</td>
<td>7.51</td>
<td>10.3</td>
</tr>
<tr>
<td>8</td>
<td>16.2</td>
<td>7.50</td>
<td>1.76</td>
<td>4.02</td>
</tr>
<tr>
<td>9</td>
<td>12.3</td>
<td>9.23</td>
<td>6.08</td>
<td>18.1</td>
</tr>
<tr>
<td>Camptothecin</td>
<td>1.95</td>
<td>0.98</td>
<td>1.69</td>
<td>2.27</td>
</tr>
</tbody>
</table>

For a compound to be deemed effective, an IC_{50} value < 100 μmol L^{-1} is required. Camptothecin was used as positive control. HL-60, human acute promyelocytic leukemia; HepG2, human hepatocellular carcinoma; KB, human oropharyngeal epidermoid carcinoma; MDA-MB-231, human breast cancer cells.

**Experimental**

**General experimental procedures**

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectra with KBr pellets. CD spectra were measured on a JASCO J-810 spectropolarimeter. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. 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% H_{2}SO_{4} in EtOH.

**Plant material**

The flower buds of *M. fargesii*, indigenous to Nanzhao county, Henang province, were purchased from Kunming Herb Medicine Market in September 2010. A voucher specimen (YNNI-10-9-28) has been deposited in our laboratory.

**Extraction and isolation**

The air-dried and powdered flower buds of *Magnolia fargesii* (4.5 kg) were extracted four times with 70% aqueous acetone (4 × 3 L) at room temperature, filtered, and the filtrate evaporated under reduced pressure and partitioned with EtOAc (4 × 3 L). The EtOAc phase (212 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 (35.8 g) by silica gel column chromatography, eluted with CHCl_{3}-acetone (9:1-2:1), yielded mixtures B1-B6. Fraction B2 (8:2, 4.8 g) was subjected to silica gel column chromatography using petroleum ether-acetone, and semi-preparative HPLC (55% MeOH-H_{2}O, flow rate 12 mL min^{-1}) to give 3 (22.6 mg), 4 (18.9 mg), 5 (22.7 mg) and 9 (28.5 mg). Fraction B3 (7:3, 5.47 g) was subjected to silica gel column chromatography using CHCl_{3}-acetone, and semi-preparative HPLC (48% MeOH-H_{2}O, flow rate 12 mL min^{-1}) to give 1 (11.6 mg) and 2 (28.9 mg). Fraction B4 (7:3, 3.28 g) was subjected to silica gel column chromatography using CHCl_{3}-acetone, and semi-preparative HPLC (40% MeOH-H_{2}O, flow rate 12 mL min^{-1}) to give 3 (14.2 mg), 6 (22.5 mg), and 7 (18.2 mg).
Anti-HIV-1 assay

The cytotoxicity assay against C8166 cells (CC50) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC50).21

Cytotoxicity assay

The cytotoxicity tests for these compounds were performed against HL-60, Hep-G2, KB, and MDA-MB-231 tumor cell lines by the MTT-assay using camptothecin as positive control.24

Fargesiphenol A (1)

Pale yellow gum; [α]D25 +38.5 (c 0.20, MeOH); CD (c 0.05, MeOH): Δε220 nm +0.36, Δε237 nm +7.18, Δε280 nm -0.97, Δε320 nm -0.21; UV (MeOH) λmax (log ε) 320 (2.82), 285 (3.78), 210 (4.27) nm; IR (KBr) νmax/cm⁻¹: 3498, 2957, 2874, 1608, 1520, 1440, 1265, 958; ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) data, Table 1; positive ESIMS m/z 397 [M+Na]+; HRESIMS m/z 397.1622 [M+Na]+ (calc. C21H26NaO6 for 397.1627).

Fargesiphenol B (2)

Yellow gum; [α]D24 +41.2 (c 0.22, MeOH); CD (c 0.05, MeOH): Δε220 nm +1.28, Δε237 nm +14.6, Δε280 nm -2.18, Δε320 nm -0.96; UV (MeOH) λmax (log ε) 320 (2.88), 282 (3.82), 210 (4.23) nm; IR (KBr) νmax/cm⁻¹: 3495, 2959, 2873, 1608, 1524, 1486, 1437, 1263, 954; ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) data, Table 1; positive ESIMS m/z 427 [M+Na]+; HRESIMS m/z 427.1730 [M+Na]+ (calc. C22H26NaO6 for 427.1733).

Fargesiphenol C (3)

Yellow gum; [α]D24 +42.5 (c 0.22, MeOH); CD (c 0.05, MeOH): Δε220 nm +0.47, Δε238 nm +6.52, Δε280 nm -0.83, Δε320 nm -0.49; UV (MeOH) λmax (log ε) 318 (2.89), 280 (3.86), 210 (4.20) nm; IR (KBr) νmax/cm⁻¹: 3493, 2950, 2876, 1614, 1529, 1482, 1442, 1260, 959; ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) data, Table 1; positive ESIMS m/z 469 [M+Na]+; HRESIMS m/z 469.1833 [M+Na]+ (calc. C24H28NaO6 for 469.1838).

Supplementary Information

¹³C NMR, DEPT, ¹H NMR, HSQC, HMBC, ¹H–¹H COSY, ROESY, CD, and HRESIMS spectra of fargesiphenol A; ¹³C NMR, DEPT, ¹H NMR, HSQC, HMBC, and CD spectra of fargesiphenol B and C, are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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References


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Figure S1. 13C NMR and DEPT spectra of fargesiphenol A (1).
Figure S2. $^1$H NMR spectra of fargesiphenol A (1).

Figure S3. HSQC spectra of fargesiphenol A (1).
Figure S4. HMBC spectra of fargesiphenol A (1).

Figure S5. $^1$H-$^1$H COSY spectra of fargesiphenol A (1).
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**Figure S6.** ROESY spectra of fargesiphenol A (1).

**Figure S7.** CD spectra of fargesiphenol A (1).
Figure S8. HRESIMS spectra of fargesiphenol A (1).

Figure S9. $^{13}$C NMR and DEPT spectra of fargesiphenol B (2).
Figure S10. $^1$H NMR spectra of fargesiphenol B (2).

Figure S11. HSQC spectra of fargesiphenol B (2).
Figure S12. HMBC spectra of fargesiphenol B (2).

Figure S13. CD spectra of fargesiphenol B (2).
Figure S14. $^{13}$C NMR and DEPT spectra of fargesiphenol C (3).

Figure S15. $^1$H NMR spectra of fargesiphenol C (3).
Figure S16. HSQC spectra of fargesiphenol C (3).

Figure S17. HMBC spectra of fargesiphenol C (3).
Figure S18. CD spectra of fargesiphenol C (3).