7,8-Secolignans from Schisandra neglecta and Their Anti-HIV-1 Activities

Xuemei Gao,²,Huaixue Mu,¹ Ruirui Wang,⁷ Qiufen Hu,*,⁷ Liemeng Yang,⁷ Yongtang Zheng,⁷ Handong Sun,² and Weilie Xiao*,¹

¹Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities, Kunming 650031, P. R. China
²State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China
³Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, P. R. China

Quatro novas 7,8-secoligninas (neglectahenols A-D), juntamente com duas 7,8-secolignanas conhecidas, foram isoladas de folhas e caules de Schisandra neglecta. As estruturas foram elucidadas por métodos espectroscópicos, incluindo a técnica de RMN (ressonância magnética nuclear) em uma e duas dimensões. As atividades anti-HIV-1 (vírus da imunodeficiência humana tipo 1) das 7,8-secoligninas e dos neglectahenols também foram testadas, e todos mostraram atividades moderadas.

Four new 7,8-secolignans (neglectahenols A-D), together with two known 7,8-secolignans, were isolated from leaves and stems of Schisandra neglecta. The structures were elucidated by spectroscopic methods, including extensive one and two dimension NMR (nuclear magnetic resonance) techniques. 7,8-secolognans and neglectahenols A-D were also tested for their anti-HIV-1 (human immunodeficiency virus type 1) activities, and all of them showed modest activities.

Keywords: Schisandra neglecta, 7,8-secolignan, anti-HIV-1 activities

Introduction

The genus Schisandra, comprising twenty three species of scandent and woody vines, is widely distributed in East Asia.¹ Fruits and stems of Schisandra plants are commonly used in traditional Chinese medicine for the treatment of hepatitis, diabetes, diarrhea and cough and used as an astringent remedy.² Phytochemically, plants of the genus Schisandra are a rich source of lignans³ and triterpenoids.⁴ Schisandra neglecta, one species of this genus, is a climbing plant mainly distributed in Southwest China. Previous chemical investigations of this plant led to the isolation of a number of dibenzocyclooctadiene lignans that showed anti-HIV-1 (human immunodeficiency virus type 1) and anti-HBV (hepatitis B virus) activities.⁵⁻⁷ In our continuous investigation of new biologically potent active compounds from the same plant, six 7,8-secolignans (1-6) were isolated, including four new ones named neglectahenols A-D (1-4). The structures of the new compounds 1-4 were established by means of mass spectrometry (MS) and extensive nuclear magnetic resonance (NMR) analyses data. In addition, the anti-HIV-1 activities of compounds 1-4 were evaluated. In this work, the isolation and structural elucidation as well as the anti-HIV activities of these compounds are reported.

Results and Discussion

A 70% aq. acetone extract prepared from leaves and stems of S. neglecta was partitioned between EtOAc and H₂O. The EtOAc layer was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC (high performance liquid chromatography) to afford compounds 1-6, including four new 7,8-secolignans named neglectahenols A-D (1-4), together with two known 7,8-secolignans, marphenol B (5)⁸ and 7,8-secohlolostylene B (6).⁹ The structures of compounds 1-6 are shown in Figure 1.

Compound 1 was obtained as a white amorphous powder, and the molecular formula C₂₁H₂₄O₄ was assigned...
by HRESIMS (high resolution electrospray ionization mass spectrometry) *m/z* 427.1372 [M + Na]^+ (calcd. 427.1369). Its 1H and 13C NMR (Table 1) spectra showed signals of 24 hydrogens and 21 carbons, respectively, corresponding to two aromatic rings (a 3', 4'-dimethoxy-5'-hydroxybenzyl and a piperonyl), one carbonyl carbon (δC 165.4), two methyl groups (δC 9.5, 22.2), one methine carbon (δC 45.1) and two oxidated methine carbons (δC 65.0, 79.2) which were in accordance with the molecular formula C22H24O6. Strong absorption bands accounting for hydroxy group (3475 cm−1), carbonyl group (1708 cm−1) and aromatic groups (1658, 1574, 1523 and 1475 cm−1) were also observed in its IR spectrum. The UV spectrum of 1 showed absorption maxima at 276 and 210 nm which confirmed the existence of the aromatic functions. In the HMBC (heteronuclear multiple-bond correlation spectroscopy) spectrum, correlations were found from H-7' (δH 7.11) to C-1' (δC 133.2), C-2' (δC 103.9) and C-6' (δC 109.7), which positioned the 3', 4'-dimethoxy-5'-hydroxyphenyl group at C-7'. The HMBC correlations of aromatic protons H-2 (δH 7.59), H-6 (δH 7.85) with C-7 (δC 165.4) suggested that another aromatic ring was attached to C-7. Moreover, the HMBC correlation of H-7' (δH 6.40) with C-7 (δC 165.4) indicated that C-7' coupled to C-7 through ester group. The above information implied that 1 should be a 7,8-secolignan.8 The spin system of H-7'/H-8'/H-8/H-9 and H-8/H-9 in the 1H-1H COSY (correlation spectroscopy) spectrum further confirmed the above deduction. By comparison, the 1H and 13C NMR spectral data of 1 are similar to those of marphenol B.8 The only difference is that the carbonyl carbon C-8 (δC 209.5) in marphenol B was replaced by an oxidated methine carbon (δC 65.0) in 1. According to the chemical shift of δC 65.0 and the molecular formula of 1, a hydroxyl group should be located at C-8. Thus, the structure of 1 was established as shown and given the name as neglectahenol A.

Compounds 2-4 (neglectahenols B-D) were all obtained as white amorphous powders. By comparison of their IR, UV, 1H and 13C NMR and ROESY (rotating frame nuclear Overhauser effect spectroscopy) spectra with those of 1, compounds 2-4 were also assigned as 7,8-secolignans. The obvious chemical shift differences resulted from the substituent group variations in the aromatic rings. Compound 2 had the molecular formula C20H22O6, as revealed by its HRESIMS data (*m/z* 397.1270 [M + Na]^+ , calcd. 397.1263). The 1H and 13C NMR spectra were similar to those of 1. Analysis of HSQC, 1H-1H-COSY and HMBC spectra of 2 showed chemical shift differences from the substituents at C-4' and C-5' in aromatic rings. A hydroxyl group replaced the methoxyl group at C-4' and no substituent at C-5' in 2 was indicated by the downfield chemical shift of C-4' (δC 148.6) and the HMBC correlations of H-5' (δH 7.11) with C-4' and C-6'. Compound 3 showed a quasi-molecular ion peak at *m/z* 457.1832 [M + Na]^+ (calcd. 457.1838) in its HRESIMS, corresponding to the molecular formula of C23H22O8. The NMR spectrum of 3 was similar to those of 1, except for the substituents at C-3, C-4 and C-5' on the two aromatic rings. A methoxyl group at C-5'

**Figure 1.** The structures of compounds 1-6.

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125.3 s</td>
<td>125.7 s</td>
<td>122.5 s</td>
<td>122.6 s</td>
<td>122.6 s</td>
<td>122.5 s</td>
<td>122.6 s</td>
<td>122.6 s</td>
<td>122.6 s</td>
<td>122.6 s</td>
</tr>
<tr>
<td>2</td>
<td>109.7 d</td>
<td>109.4 d</td>
<td>113.5 d</td>
<td>113.6 d</td>
<td>113.6 d</td>
<td>113.6 d</td>
<td>113.6 d</td>
<td>113.6 d</td>
<td>113.6 d</td>
<td>113.6 d</td>
</tr>
<tr>
<td>3</td>
<td>148.3 s</td>
<td>148.0 s</td>
<td>150.6 s</td>
<td>151.0 s</td>
<td>151.0 s</td>
<td>151.0 s</td>
<td>151.0 s</td>
<td>151.0 s</td>
<td>151.0 s</td>
<td>151.0 s</td>
</tr>
<tr>
<td>4</td>
<td>152.3 s</td>
<td>153.6 s</td>
<td>154.6 s</td>
<td>154.7 s</td>
<td>154.7 s</td>
<td>154.7 s</td>
<td>154.7 s</td>
<td>154.7 s</td>
<td>154.7 s</td>
<td>154.7 s</td>
</tr>
<tr>
<td>5</td>
<td>108.4 d</td>
<td>108.1 d</td>
<td>111.3 d</td>
<td>111.7 d</td>
<td>111.7 d</td>
<td>111.7 d</td>
<td>111.7 d</td>
<td>111.7 d</td>
<td>111.7 d</td>
<td>111.7 d</td>
</tr>
<tr>
<td>6</td>
<td>125.7 d</td>
<td>124.6 d</td>
<td>124.5 d</td>
<td>124.8 d</td>
<td>124.8 d</td>
<td>124.8 d</td>
<td>124.8 d</td>
<td>124.8 d</td>
<td>124.8 d</td>
<td>124.8 d</td>
</tr>
<tr>
<td>7</td>
<td>165.4 s</td>
<td>165.9 s</td>
<td>165.9 s</td>
<td>165.5 s</td>
<td>165.5 s</td>
<td>165.5 s</td>
<td>165.5 s</td>
<td>165.5 s</td>
<td>165.5 s</td>
<td>165.5 s</td>
</tr>
<tr>
<td>8</td>
<td>65.0 d</td>
<td>65.0 d</td>
<td>65.1 d</td>
<td>65.0 d</td>
<td>65.0 d</td>
<td>65.0 d</td>
<td>65.0 d</td>
<td>65.0 d</td>
<td>65.0 d</td>
<td>65.0 d</td>
</tr>
<tr>
<td>9</td>
<td>22.2 q</td>
<td>22.4 q</td>
<td>22.6 q</td>
<td>21.8 q</td>
<td>21.8 q</td>
<td>21.8 q</td>
<td>21.8 q</td>
<td>21.8 q</td>
<td>21.8 q</td>
<td>21.8 q</td>
</tr>
<tr>
<td>1'</td>
<td>133.2 s</td>
<td>134.3 s</td>
<td>133.7 s</td>
<td>133.7 s</td>
<td>133.7 s</td>
<td>133.7 s</td>
<td>133.7 s</td>
<td>133.7 s</td>
<td>133.7 s</td>
<td>133.7 s</td>
</tr>
<tr>
<td>2'</td>
<td>103.9 d</td>
<td>113.5 d</td>
<td>105.6 d</td>
<td>103.9 d</td>
<td>103.9 d</td>
<td>103.9 d</td>
<td>103.9 d</td>
<td>103.9 d</td>
<td>103.9 d</td>
<td>103.9 d</td>
</tr>
<tr>
<td>3'</td>
<td>154.0 s</td>
<td>154.0 s</td>
<td>153.5 s</td>
<td>153.7 s</td>
<td>153.7 s</td>
<td>153.7 s</td>
<td>153.7 s</td>
<td>153.7 s</td>
<td>153.7 s</td>
<td>153.7 s</td>
</tr>
</tbody>
</table>

**Table 1.** 13C NMR data of compounds 1-4 (δ ppm, 125 MHz, CD3N)
The results showed that 1-4 exhibited therapeutic index (TI) values of 58.65, 26.36, 23.71 and 25.85, respectively. The TI values of all compounds were higher than those of reported 7,8-seco-7,8-secolignans, marphenol A, marphenol B and 7,8-seco-7,8-secolignans, marphenol A, marphenol B and 7,8-seco-7,8-secolignans. The results indicated that the reduction of keto-carbonyl to hydroxyl group at C-8 may enhance the anti-HIV activity of these compounds.

### Table 2. Anti-HIV activities of the 7,8-secolignans

<table>
<thead>
<tr>
<th>Compound</th>
<th>CC_{50} (μg mL⁻¹)</th>
<th>EC_{50} (μg mL⁻¹)</th>
<th>TI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>186.5</td>
<td>3.18</td>
<td>58.65</td>
</tr>
<tr>
<td>2</td>
<td>111.3</td>
<td>4.22</td>
<td>26.36</td>
</tr>
<tr>
<td>3</td>
<td>91.8</td>
<td>3.87</td>
<td>23.71</td>
</tr>
<tr>
<td>4</td>
<td>107.0</td>
<td>4.14</td>
<td>25.85</td>
</tr>
<tr>
<td>AZT</td>
<td>&gt; 200</td>
<td>0.029</td>
<td>&gt; 6896.55</td>
</tr>
</tbody>
</table>

CC_{50}: median cytotoxic concentration; EC_{50}: concentration for 50% of maximal effect; TI: CC_{50}/EC_{50}

### 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 IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS (tetramethylsilane) 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 C₁₈ (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with silica 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 (thin layer chromatography), and spots were visualized by heating Si gel plates sprayed with 5% H₂SO₄ in EtOH.

### Plant material

The leaves and stems of *S. neglecta* were collected in Lijiang Country, Yunnan Province, People’s Republic of China.
of China, in September 2009. The identification of the plant material was verified by Prof. Xi-Wen Li of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KIB 09-9-34) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered leaves and stems of *S. neglecta* (4.0 kg) were extracted four times with 70% aqueous Me$_2$CO (4 × 5 L) at room temperature and filtered, with the filtrate evaporated under reduced pressure and partitioned with EtOAc (4 × 5 L). The EtOAc partition (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, 6:4, 5:5), to give six fractions A-F. The further separation of fraction B (9:1, 52.5 g) by silica gel column chromatography, eluted with CHCl$_3$:acetone (20:1-2:1), yielded mixtures A1-A7. Fraction A2 (9:1, 36 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (65% MeOH-H$_2$O, flow rate 12 mL min$^{-1}$) to give 5 (17.2 mg) and 6 (21.4 mg). Fraction A3 (8:2, 15.8 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (60% MeOH-H$_2$O, flow rate 12 mL min$^{-1}$) to give 1 (16.4 mg), 2 (8.64 mg), 3 (10.6 mg) and 4 (8.25 mg).

Anti-HIV-1 assay

The cytotoxicity assay against C8166 cells (CC$_{50}$ concentration for 50% of maximal effect) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC$_{50}$).$^{10}$

**Neglectahenol A (1)**

White amorphous powder; [α]$^{26.5}_{D}$ +22.6 (c 0.22, MeOH); UV (MeOH) $\lambda_{max}$/nm (log ε): 210 (4.26), 276 (3.88), 340 (2.78); IR (KBr) $\nu_{max}$/cm$^{-1}$: 3475, 3065, 2938, 2854, 1708, 1658, 1574, 1523, 1475, 1392, 1345, 1258, 1135, 1027, 982, 876; 1H NMR (500 MHz, CD$_3$N) $\delta$: 7.59 (1H, d, J 1.8 Hz, H2), 6.87 (1H, d, J 8.2 Hz, H5), 7.85 (1H, dd, J 1.8, 8.2 Hz, H6), 4.63 (1H, m, H8), 1.41 (3H, d, J 6.4 Hz, H9), 6.91 (1H, s, H2'), 7.27 (1H, s, H6'), 6.40 (1H, d, J 10.3 Hz, H7'), 2.36 (1H, m, H8'), 1.05 (3H, d, J 6.6 Hz, H9'), 3.82 (3H, s, MeO-3'), 3.75 (3H, s, MeO-4'), 5.99, 6.01 (2H, s, –OCH$_2$O–); 13C NMR data, Table 1; positive ESIMS $m/z$ 427 [M + Na]$^+$; HRESIMS $m/z$ 427.1372 [M + Na]$^+$, (calcd. C$_{26}$H$_{32}$NaO$_8$ for 427.1369).

**Neglectahenol B (2)**

White amorphous powder; [α]$^{26.5}_{D}$ +18.4 (c 0.20, MeOH); UV (MeOH) $\lambda_{max}$/nm (log ε): 210 (4.19), 275 (3.86), 340 (2.82); IR (KBr) $\nu_{max}$/cm$^{-1}$: 3482, 3047, 2932, 2875, 1712, 1655, 1577, 1526, 1474, 1397, 1344, 1261, 1130, 1024, 987, 874; 1H NMR (500 MHz, CD$_3$N) $\delta$: 7.78 (1H, d, J 1.8 Hz, H2), 6.89 (1H, d, J 8.1 Hz, H5), 7.89 (1H, dd, J 1.8, 8.2 Hz, H6), 4.64 (1H, m, H8), 1.42 (3H, d, J 6.4 Hz, H9), 7.35 (1H, d, J 1.8 Hz, H2'), 7.11 (1H, d, J 8.1 Hz, H5'), 7.25 (1H, dd, J 1.8, 8.1 Hz, H6'), 6.37 (1H, d, J 10.4 Hz, H7'), 2.37 (1H, m, H8'), 1.01 (3H, d, J 6.8 Hz, H9'), 3.88 (3H, s, Me-O-3'), 5.94, 5.96 (2H, s, –OCH$_2$O–), 11.01 (1H, s, Ar–H); 13C NMR data, Table 1; positive ESIMS $m/z$ 397 [M + Na]$^+$; HRESIMS $m/z$ 397.1270 [M + Na]$^+$, (calcd. C$_{26}$H$_{30}$NaO$_8$ for 397.1263).

**Neglectahenol C (3)**

White amorphous powder; [α]$^{26.5}_{D}$ +18.9 (c 0.21, MeOH); UV (MeOH) $\lambda_{max}$/nm (log ε): 210 (4.21), 275 (3.93), 340 (2.76); IR (KBr) $\nu_{max}$/cm$^{-1}$: 3486, 3042, 2935, 2871, 1716, 1653, 1579, 1554, 1525, 1470, 1394, 1345, 1266, 1134, 1058, 1020, 980, 877; 1H NMR (500 MHz, CD$_3$N) $\delta$: 7.44 (1H, d, J 1.8 Hz, H2), 7.04 (1H, d, J 8.2 Hz, H5), 7.66 (1H, dd, J 1.8, 8.2 Hz, H6), 4.47 (1H, m, H8), 1.33 (3H, d, J 6.4 Hz, H9), 6.48 (2H, s, H-2', H6'), 5.33 (1H, d, J 10.3 Hz, H7'), 2.55 (1H, m, H8'), 1.06 (3H, d, J 6.8 Hz, H9'), 3.68 (3H, s, Me-O-3), 3.80 (3H, s, Me-O-4), 3.87 (3H, s, Me-O-3'), 3.80 (3H, s, Me-O-4'), 3.90 (3H, s, Me-O-5'); 13C NMR data, Table 1; positive ESIMS $m/z$ 457 [M + Na]$^+$; HRESIMS $m/z$ 457.1832 [M + Na]$^+$, (calcd. C$_{29}$H$_{37}$NaO$_8$ for 457.1838).

**Neglectahenol D (4)**

White amorphous powder; [α]$^{26.5}_{D}$ +16.9 (c 0.20, MeOH); UV (MeOH) $\lambda_{max}$/nm (log ε): 210 (4.15), 275 (3.87), 340 (2.69); IR (KBr) $\nu_{max}$/cm$^{-1}$: 3483, 3039, 2930, 2874, 1713, 1656, 1575, 1526, 1467, 1398, 1351, 1269, 1137, 1038, 1027, 979, 872; 1H NMR (500 MHz, CD$_3$N) $\delta$: 7.49 (1H, d, J 1.8 Hz, H2), 7.02 (1H, d, J 8.2 Hz, H5), 7.67 (1H, dd, J 1.8, 8.2 Hz, H6), 4.63 (1H, m, H8), 1.42 (3H, d, J 6.4 Hz, H9), 6.89 (1H, s, H2'), 7.30 (1H, s, H6'), 6.37 (1H, d, J 10.3 Hz, H7'), 2.36 (1H, m, H8'), 1.03 (3H, d, J 6.8 Hz, H9'), 3.81 (3H, s, Me-O-3), 3.86 (3H, s, Me-O-4), 3.96 (3H, s, Me-O-3'), 3.85 (3H, s, Me-O-4'), 11.10 (1H, bs, Ar–OH); 13C NMR data, Table 1; positive ESIMS
m/z 443 [M + Na]+; HRESIMS m/z 443.1675 [M + Na]+, (calcd. C_{22}H_{36}NaO_{8} for 443.1682).

Supplementary Information

\(^{13}C\) and \(^{1}H\) NMR and DEPT spectra of neglectahenols A-D and HSQC, HMBC, \(^{1}H-^{1}H\) COSY, ROESY and HRESIMS spectra of neglectahenol A are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

This project was financially supported by the NSFC (30830115) and the project from the Chinese Academy of Sciences (KSCX2-EW-Q-10).

References


Submitted: February 2, 2012
Published online: October 11, 2012