Sesquiterpenoids and Lignans from the Roots of *Syringa pinnatifolia*

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Two new sesquiterpenoids, pinnatifone A (1) and pinnatifone B (2), and two new lignans, pinnatifolin (3) and isopinnatifolin (4), along with six known lignans (5–10), were isolated from the roots of *Syringa pinnatifolia*. The structures of the new compounds were elucidated by extensive spectroscopic methods, including NMR, MS, UV, and IR spectra. The lignans were screened for their anti-oxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH) assay). Most of them showed potent anti-oxidant activity, especially compound 5, whose potent anti-oxidant activity had an SC50 value higher than that of the positive control vitamin C.

**Key words** *Syringa pinnatifolia*; Oleaceae; sesquiterpenoid; lignan; anti-oxidant activity; 2,2-diphenyl-1-picrylhydrazyl (DPPH)

*Syringa pinnatifolia* HEMSCL., a member of the family Oleaceae, is distributed predominantly in spinney and shrub of Helan Mountain, Inner Mongolia. The stem and root of this plant, known as “Shan Chenxiang” in Chinese, is often used as a traditional herbal medicine for the treatment of cardiovascular and respiratory diseases in China and Mongolia. The stem and root of this plant, which is called “Chenxiang” in Chinese, is distributed predominantly in spinney and shrub of Helan Mountain, Inner Mongolia. The stem and root of this plant, known as “Shan Chenxiang” in Chinese, is often used as a traditional herbal medicine for the treatment of cardiovascular symptoms, pain, asthma, and heat in Mongolian medicine.1–4 “Shan Chenxiang” is also widely distributed in Mongolia as a substitute of the precious traditional medicinal material—Lignum Aquilariae Resinatum, which is called “Chenxiang” in Chinese. Previous investigations have shown that the plant mainly contained lignans5–7 and essential oil.8,9 In this research, two new sesquiterpenoids, pinnatifone A (1), pinnatifone B (2) and two new lignans, pinnatifolin (3), isopinnatifolin (4), along with six known ones (5–10), were isolated from the stems of *Syringa pinnatifolia* (Fig. 1). In this paper, we describe the isolation and structural elucidation of the new compounds, as well as the anti-oxidant activity of the lignans isolated from this plant.

**Results and Discussion**

Compound 1 was isolated as a white crystal. The molecular formula was determined as C15H18O3 by high resolution-electrospray ionization-mass spectrometry (HR-ESI-MS) ion peak at m/z 249.1488 [M+H]+ (Calcd. for C15H18O3, 249.1485). The IR spectrum showed the absorption bands of hydroxyl group at 3399 cm−1 and carbonyl group at 1669 cm−1. The 1H-NMR spectrum (Table 1) revealed three methyls at δH 1.36 (3H, s), 1.75 (3H, s) and 1.72 (3H, s), two olefinic protons at δH 4.77 (1H, s) and 4.71 (1H, s). The 13C-NMR spectrum of 1 showed the presence of 15 carbon signals, including four olefinic carbons at δC 110.4, 146.7, 144.6 and 129.6, and two carbonyl carbons at δC 209.0 and 191.6. All the above data suggested 1 was a sesquiterpenoid. Comparison of the 13C-NMR data of 1 with those of the known compound (3R,4aS,8aR)-3,4,4a,8a-tetrahydro-6-hydroxy-5,8a-dimethyl-1-(1-methylethenyl)-1,7-naphthalenenedione(10) displayed that most of the 13C-NMR signals of the two compounds were almost same, except the signals at δC 54.4 (C-8a), 32.0 (C-8), 27.1 (C-12) and 10.8 (C-13) in 1 had a few differences from the signals of δC 49.7, 29.3, 24.4, 13.9 in the compared compound, suggesting that C-8a, C-8, C-12 and C-13 did not have the same chemical environment as the compared compound. The 1H–1H correlation spectroscopy (COSY) cross-peaks of H-8a (δH 2.66)/H-8 (δH 3.01), H-3 (δH 1.60)/H-2 (δH 2.29) and H-4 (δH 2.21), and the heteronuclear multiple bond connectivity (HMBC) correlations from H-12 (δH 1.36) to C-8a (δC 54.4), C-4a (δC 42.9), C-4 (δC 42.1) and C-5 (δC 129.6), indicated that a methyl (C-12) was connected to C-4a. The rotating frame Overhauser enhancement spectroscopy (ROESY) correlation between H-12 (δH 1.36) and H-8a (δH 2.66) assigned that H-12 and H-8a were on the same side. Furthermore, the relative structure of 1 was confirmed by a single-crystal X-ray diffraction study (Fig. 2). Thus, the structure of 1 was elucidated and named as pinnatifone A.

The molecular formula of compound 2 was determined to be C15H18O3 by HR-ESI-MS at m/z 269.1149 [M+Na]+ (Calcd. for C15H18O3NaO, 269.1154). The 1H- and 13C-NMR data of 2 were very similar to those of compound 1, except the signals at δH 46.0 (C-2) and 41.2 (C-3) in 1 were very different from the signals at δC 135.6 and 159.0 in 2, suggesting that 2 had a double bond at C-2 and C-3. This was further confirmed by HMBC correlations from H-2 (δH 6.45) to C-1 (δC 201.3), C-3 (δC 159.0), C-4 (δC 38.8) and C-9 (δC 146.8). The ROESY correlation of H-12 (δH 1.32)/H-8a (δH 2.11) and assigned the relative configuration of 2. Thus, compound 2 was elucidated and named as pinnatifone B.

Compound 3 was obtained as colorless oil. Its molecular formula of C20H23O5 was deduced from the HR-ESI-MS ion peak at m/z 343.1540 [M+H]+ (Calcd. for C20H23O5, 343.1540). The 13C-NMR data of 3 were very similar to those of (-)laliciresinol (6), except the signals at C-8 (δC 54.1) and C-9 (δC 60.6) in compound 6 changed to be a double bond (δC 154.8 and 107.7) in 3, suggesting that 3 had a double bond at C-8 and C-9. The ROESY correlation between H-8’ (δH 3.05) and H-7 (δH 5.19) indicated that H-8’ and H-7 were on the same side, which assigned the relative configuration of 3. Hence, compound 3 was elucidated and named as pinnatifolin.

Compound 4 was obtained as colorless oil and assigned the
same molecular formula C_{20}H_{22}O_{5} as that of 3 on the basis of its HR-ESI-MS at m/z 343.1541 ([M+H]+, Calcd for C_{20}H_{23}O_{5}, 343.1540). Compound 4 exhibited similar UV, IR spectra as those of 3 except that the NMR spectra had some differences at C-8, C-8 and C-7. Detailed analysis of the two dimensional (2D)-NMR spectra indicated that 4 had a same planar structure as 3. In the ROESY spectrum, H-7 (δ_{H} 2.99 and 2.59) showed correlation with H-7 (δ_{H} 5.20), and no correlation between H-8 (δ_{H} 3.11) and H-7, indicating that H-8 and H-7 were on the opposite side. Hence, compound 4 was elucidated and named as isopinnatifolin.

Compounds 5–10 were identified as (−)-secoisolariciresinol, (−)-lariresinol, (8R,8′R,9R)-4,4′-dihydroxy-3,3′,9-trimethoxy-9,9′-epoxylignan, (8R,8′R,9S)-4,4′-dihydroxy-3,3′,9′-trimethoxy-9,9′-epoxylignan, (8R,8′R,9R)-4,4′-dihydroxy-3,3′,9′-trimethoxy-9,9′-epoxylignan, (8R,8′R,9R)-4,4′,9-trihydroxy-3,3′-dimethoxy-9,9′-epoxylignan, respectively, by comparing their spectral data with literature values.\(^\text{11–15}\)

Oxidant stress plays an important role in the pathogenesis of many cardiovascular diseases (CVDs), and the use of antioxidant supplements is very beneficial to the prevention of coronary artery diseases.\(^\text{16}\) Due to the bioactivity of S. pinnatifolia related to the cardiovascular system, all the lignans isolated from this plant in our present studies were tested for their antioxidant activity. As shown in Table 3, most of the lignans showed potent antioxidant activity. Especially, compound 5 possessed potent antioxidant activity with SC_{50} value of

| Position | \(\delta_{C}\) | \(\delta_{H}\) | \(\delta_{C}\) | \(\delta_{H}\) |
|----------|-------------|-------------|-------------|-------------|
| 1        | 209.0       | —           | 201.1       | —           |
| 2        | 46.0        | 2.43 (dd, 16.8, 6.0) | 135.6       | 6.45 (s)   |
|          |             | 2.29 (br d, 16.8) |             |             |
| 3        | 41.2        | 1.60 (m)    | 159.0       | —           |
| 4        | 42.1        | 2.21 (d, 16.0) | 38.8        | 2.00 (s)   |
| 5        | 129.6       | —           | 124.7       | —           |
| 6        | 144.6       | —           | 144.1       | —           |
| 7        | 191.6       | —           | 191.6       | —           |
| 8        | 32.0        | 3.01 (dd, 17.2, 12.0) | 38.0        | 2.92 (dd, 18.0, 12.0) |
|          |             | 2.57 (dd, 17.2, 2.8) |             | 2.42 (dd, 18.0, 4.8) |
| 9        | 146.7       | —           | 146.8       | —           |
| 10       | 110.4       | 4.77 (s) 4.71 (s) | 110.8       | 4.78 (s)   |
| 4a       | 42.9        | —           | 42.1        | —           |
| 8a       | 54.4        | 2.66 (dd, 2.8, 2.0) | 41.4        | 2.11 (dd, 12.0, 4.8) |
| 11       | 20.5        | 1.72 (s)    | 20.2        | 1.92 (s)   |
| 12       | 27.1        | 1.36 (s)    | 28.5        | 1.32 (s)   |
| 13       | 10.8        | 1.75 (s)    | 11.4        | 1.74 (s)   |

\(a\) 1 was measured in CDCl_{3}, \(^1\)H-NMR at 400 MHz and \(^13\)C-NMR at 100 MHz, 2 was measured in DMSO-\(d_6\), \(^1\)H-NMR at 300 MHz and \(^13\)C-NMR at 75 MHz.

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**Fig. 1. Chemical Structures of 1–10**

**Fig. 2. Single X-Ray Diffraction and Key HMBC Correlations of 1**

Table 1. \(^1\)H- and \(^13\)C-NMR Data of 1 and 2 (\(\delta\) in ppm, \(J\) in Hz)

| Position | \(\delta_{C}\) | \(\delta_{H}\) | \(\delta_{C}\) | \(\delta_{H}\) |
|----------|-------------|-------------|-------------|-------------|
| 1        | 209.0       | —           | 201.1       | —           |
| 2        | 46.0        | 2.43 (dd, 16.8, 6.0) | 135.6       | 6.45 (s)   |
|          |             | 2.29 (br d, 16.8) |             |             |
| 3        | 41.2        | 1.60 (m)    | 159.0       | —           |
| 4        | 42.1        | 2.21 (d, 16.0) | 38.8        | 2.00 (s)   |
| 5        | 129.6       | —           | 124.7       | —           |
| 6        | 144.6       | —           | 144.1       | —           |
| 7        | 191.6       | —           | 191.6       | —           |
| 8        | 32.0        | 3.01 (dd, 17.2, 12.0) | 38.0        | 2.92 (dd, 18.0, 12.0) |
|          |             | 2.57 (dd, 17.2, 2.8) |             | 2.42 (dd, 18.0, 4.8) |
| 9        | 146.7       | —           | 146.8       | —           |
| 10       | 110.4       | 4.77 (s) 4.71 (s) | 110.8       | 4.78 (s)   |
| 4a       | 42.9        | —           | 42.1        | —           |
| 8a       | 54.4        | 2.66 (dd, 2.8, 2.0) | 41.4        | 2.11 (dd, 12.0, 4.8) |
| 11       | 20.5        | 1.72 (s)    | 20.2        | 1.92 (s)   |
| 12       | 27.1        | 1.36 (s)    | 28.5        | 1.32 (s)   |
| 13       | 10.8        | 1.75 (s)    | 11.4        | 1.74 (s)   |
Table 3. 1H- and 13C-NMR Data of 3 and 4 (δ in ppm, J in Hz)*

| Position | δ_C | δ_H  |
|----------|-----|------|
| 3        |     |      |
| 4        |     |      |

18.32±1.66 µM, comparable to the positive control vitamin C.

**Experimental**

**General Experimental Procedures** Melting points were obtained on an X-5 micro-melting point apparatus (Tech, Beijing, P.R. China). Optical rotation values were measured on a Jasco FT/IR-480 Plus spectrometer. UV spectra were recorded on a Jasco V-550 UV/VIS instrument. A Jasco FTIR-480 Plus spectrometer was used for scanning the IR spectra with KBr pellets. 1D- and 2D-NMR spectra were recorded on a Bruker spectrometer. Table 3. The SC 50 Values in µM of Anti-oxidant Activities of 3–10

| Compounds | SC 50 (µM) |
|-----------|------------|
| 3         | 85.22±1.10 |
| 4         | 43.95±0.95 |
| 5         | 18.32±1.66 |
| 6         | 25.82±1.56 |
| 7         | 25.65±2.77 |
| 8         | 26.29±2.68 |
| 9         | 25.04±0.23 |
| 10        | 24.92±0.51 |

Vitamin C (positive control) 18.65±1.90 µM, comparable to the positive control vitamin C. Yantai Chemical Industrial Institute, Yantai, China. Preparative HPLC was performed on an Agilent system equipped with a preparative Cosmolsil C18 (5 µm, 20×250 mm) column.

**Plant Materials** The roots of *S. pinnatifolia* were collected in Helan Mountain, Inner Mongolia, China, in July of 2011, and were authenticated by Prof. Guang-Xiong Zhou of Jinan University. A voucher specimen (No. 2011071013) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, P.R. China.

**Extraction and Isolation** The air-dried roots of *S. pinnatifolia* (8 kg) were extracted with 95% EtOH for three times, and the extracts were combined and concentrated under reduced pressure at 55°C to yield 320 g of crude extract. The EtOH extract was dissolved in H2O and then extracted successively with petroleum ether, ethyl acetate (EtOAc). The EtOAc extract (213 g) was chromatographed on silica gel column (18 cm×70 cm) with eluents of petroleum ether–EtOAc (0:100) to yield 3 (26.9 mg) and 2 (28.0 mg). Fraction 13 (9 g) was rechromatographed on silica gel column (4 cm×50 cm, CHCl3–MeOH, 100:1–70:30) to yield subfractions 13-1 to 13-7. Subfraction 13-3 (1.2 g) was further purified over Sephadex LH-20 (MeOH) to afford subfractions 13-1 (25 mg) and 13-2 (13.0 mg), which were separated by preparative HPLC (60% MeOH–H2O) on a Cosmosil C18 column (5 µm, 20×250 mm). Fraction 15 (6 g) was separated by preparative RP-HPLC (58% MeOH–H2O) on a Cosmosil C18
column (5 μm, 20×250 mm) to yield 3 (11.5 mg), 4 (8.6 mg), 8 (16.5 mg). Fraction 18 (7 g) was separated by the same procedure as fraction 15 and 7 (12.0 mg), 9 (8.2 mg), 10 (16.5 mg) were obtained finally.

Pinnatifone A (1) White crystals (MeOH); mp 114.0–115.8°C; [α]D 25 32.38 (c=0.1, CHCl3); UV λmax (MeOH) nm: 202, 274; IR νmax (KBri) cm−1: 3399, 2929, 1675, 1392, 895; HR-ESI-MS m/z 249.1488 [M+H]+ (Calcd for C15H20O3: 249.1485); 1H- and 13C-NMR data, see Table 1.

Pinnatifolin (2) White powder (MeOH); [α]D 25 5+11.12 (c=0.1, CHCl3); UV λmax (MeOH) nm: 203, 280; IR νmax (KBri) cm−1: 3416, 2923, 1605, 1516, 1456, 1268, 1031, 929, 802; HR-ESI-MS m/z 343.1540 [M+H]+ (Calcd for C20H23O5: 343.1540); 1H- and 13C-NMR data, see Table 1.

Pinnatifoline B (3) Colorless oil; C20H22O5; [α]D 25 25−26.76 (c=0.1, MeOH); UV λmax (MeOH) nm: 207, 237, 281; IR νmax (KBri) cm−1: 3426, 2923, 1605, 1516, 1456, 1281, 1039, 929, 802; HR-ESI-MS m/z 343.1540 [M+H]+ (Calcd for C20H23O5: 343.1540); 1H- and 13C-NMR data, see Table 2.

Isopinnatifolin (4) Colorless oil; [α]D 25 15.26 (c=0.1, MeOH); UV λmax (MeOH) nm: 207, 237, 281; IR νmax (KBri) cm−1: 3429, 2921, 1616, 1523, 1496, 1244, 1034, 928, 827; HR-ESI-MS m/z 343.1541 [M+H]+ (Calcd for C20H23O5: 343.1540); 1H- and 13C-NMR data, see Table 2.

X-Ray Crystallography of Compound 1 The X-ray data of compound 1 were collected at 293(2) K on a Rigaku Mercury CCD diffractometer. Crystal data: C15H21O3 (fw 252.31). The final reliability factors were: R=0.0774, Rw=0.2248, and the goodness of fit on F2 was equal to 1.144. Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 951468.

2.2-Diphenyl-1-picyrylhydrozyl (DPPH) Radical Scavenging Assay The DPPH radical scavenging assay was carried out according to Gao et al.18) and Zhang et al.19) Briefly, 100 μL of DPPH solution (200 μM in ethanol) was added to a 96-well microplate containing 100 μL of 2-fold serial dilutions of each sample (in ethanol, with amounts of samples ranging from 0 to 500 μM). The mixture was vortexed for 1 min and then incubated for 30 min in the dark at room temperature. The absorbance of the reaction mixtures was recorded at 517 nm by using a multi-mode detection microplate reader (Bio-Tek, Synergy™2, U.S.A.). The DPPH scavenging activity was calculated by the following equation: % scavenging activity=100×(Acontrol−A sample)/Acontrol. The percentage of the DPPH reduction was plotted against the sample concentration to calculate the SC50, which was the concentration of sample needed to scavenge 50% of DPPH radical. The purities (%) of tested compounds were above 95%. All samples were measured in triplicate, with Vitamin C (Sigma, purity≥99%) as the positive control, and the data represents means±S.D.s of three determinations.

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