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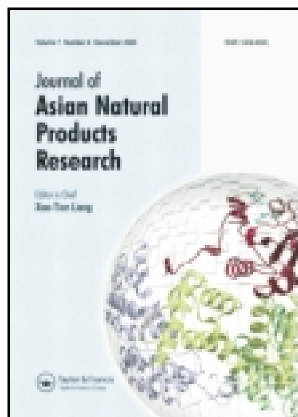


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# Lignans from *Schisandra lancifolia*

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Two new neolignans, schilancifolignans D and E (**1** and **2**), together with eight known ones, were isolated from the leaves and stems of *Schisandra lancifolia*. The structures of **1** and **2** were elucidated by spectroscopic methods, including extensive 1D and 2D NMR techniques. Compounds **1** and **2** were tested for their anti-HIV-1 activities and cytotoxicity. Both showed significant potential cytotoxic ability and weak anti-HIV-1 activities.

**Keywords:** *Schisandra lancifolia*; lignans; schilancifolignans D and E; anti-HIV-1 activities; cytotoxicity

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## 1. Introduction

Plants of the economically and medicinally important genus *Schisandra* (Schisandreae) are known to be a rich source of lignans, lanostane, and cycloartane triterpenes, which have been found to possess various beneficial pharmacological effects [1,2]. Since 2003, the systematical chemical investigation of the genus *Schisandra* conducted by our group led to the discovery of a series of novel nortriterpenoids with a diversity of highly oxygenated structures biogenetically related to cycloartane, some of which showed promising anti-HIV-1 activities with low toxicities [2–5]. *Schisandra lancifolia*, one species of this genus, is a climbing plant mainly distributed in Mainland of China [6]. In our previous work, some new highly oxygenated nortriterpenoids and dibenzocyclooctadiene lignans were isolated from this plant [7–9]. As a part of our search for bioactive materials, the phytochemical study of an EtOAc extract of *S. lancifolia* led to the isolation of two neolignans (**1** and **2**), along with eight known compounds (**3**–**10**). The structural elucidation of compounds **1** and **2** and the evaluation of their anti-HIV-1 activities and cytotoxicity in several cancer cell lines are reported in this study.

## 2. Results and discussion

A 70% aq. acetone extract prepared from the leaves and stems of *S. lancifolia* was partitioned between EtOAc and $H_2O$. The EtOAc layer was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1**–**10** (Figure 1), including two new neolignans named schilancifolignans D and E (**1** and **2**), together with eight known lignans, pregomisin (**3**) [10], 4-[(2*R*,3*S*)-4-(3,4-dimethoxyphenyl)-2,3-dimethylbutyl]-2-methoxy-phenol (**4**) [11], 4,4′-(2*R*,3*S*)-2,3-

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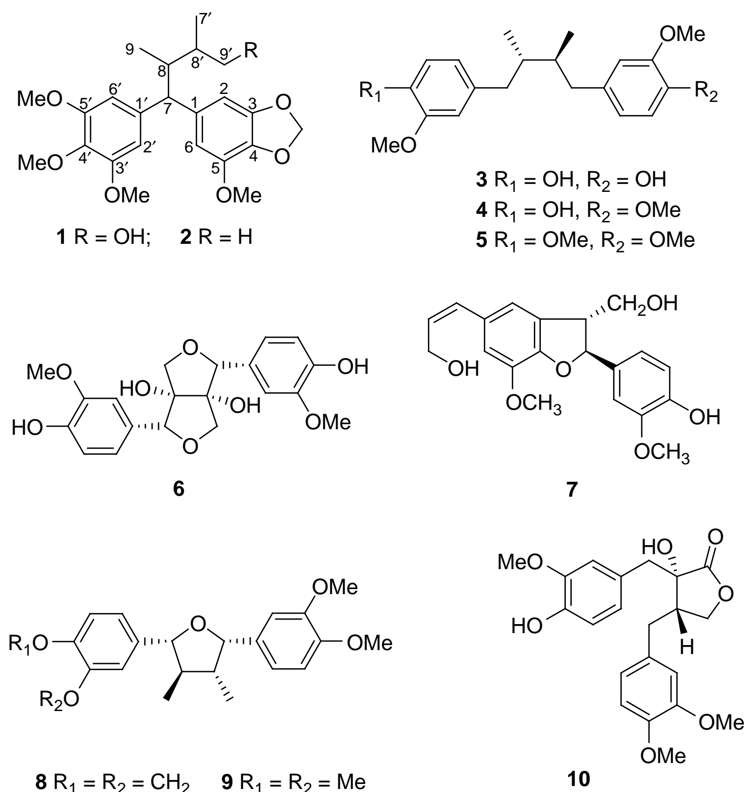


Figure 1. The structures of compounds **1**–**10**.

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dimethylbutane-1,4-diyl)bis(1,2-dimethoxybenzene) (**5**) [12], prinsepiol (**6**) [13], dehydrodiconiferyl alcohol (**7**) [14], austrobailignan (**8**) [15], dimethyltetrahydrofuroguaiacin (**9**) [16], and wik-stromol (**10**) [17].

Compound **1** was obtained as a yellow gum. Its molecular formula was determined as $C_{23}H_{30}O_7$ by HR-ESI-MS at *m/z* 441.1895 $[M + Na]^+$. Its $^1H$ and $^{13}C$ NMR spectra (Table 1) showed signals of 30 protons and 23 carbons, respectively, corresponding to two aromatic rings with four aromatic protons, two methyl groups, four methoxyl groups, one methylenedioxy group, one oxidated methylene group, and three methine signals. Strong absorption bands accounting for hydroxy ($3340\,cm^{-1}$) and aromatic groups (1610, 1595, 1518, and $1460\,cm^{-1}$) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 282 and 205 nm, which confirmed the existence of the aromatic functions. The HMBC spectrum of compound **1** showed cross-peaks between H-7 ($\delta_H$ 3.99, d, $J = 11.4$ Hz) and carbons of both aromatic rings, C-2 ($\delta_C$ 104.6), C-6 ($\delta_C$ 107.2), C-2′ ($\delta_C$ 106.6), C-6′ ($\delta_C$ 106.6), whereas there was no correlation between H-9′ and any aromatic carbon, which indicated that the two aromatic rings were linked to the same carbon C-7. The $^1H-^1H$ COSY correlations of H-7/H-8/H-8′/H-9′, H-8/H-9, and H-8′/H-7, together with HMBC correlations (Figure 2) of H-7 ($\delta_H$ 3.99, d, $J = 11.4$ Hz) with C-8 ($\delta_C$ 36.7), C-9 ($\delta_C$ 12.2), and C-8′ ($\delta_C$ 36.6), and of H-7′ ($\delta_H$ 0.94, d, $J = 7.1$ Hz) with C-8′ ($\delta_C$ 36.6), C-9′ ($\delta_C$ 66.8), C-8 ($\delta_C$ 36.7),

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** ($\delta$ in ppm, data obtained in pyridine-$d_5$).

| | **1** | | **2** | |
|---|---|---|---|---|
| No. | $^{13}$C | $^{1}$H | $^{13}$C | $^{1}$H |
| 1 | 137.2, s | | 137.5, s | |
| 2 | 104.6, d | 6.46, s | 104.9, d | 6.47, s |
| 3 | 148.5, s | | 148.8, s | |
| 4 | 133.9, s | | 133.8, s | |
| 5 | 146.1, s | | 146.6, s | |
| 6 | 107.2, d | 6.35, s | 107.5, d | 6.37, s |
| 7 | 56.6, d | 3.99, d, $J = 11.4$ Hz | 57.5, d | 4.07, d, $J = 11.6$ Hz |
| 8 | 36.7, d | 3.19–3.22, m | 39.6, d | 3.32–3.36, m |
| 9 | 12.2, q | 0.91, d, $J = 6.9$ Hz | 15.0, q | 1.23, d, $J = 6.7$ Hz |
| 10 | 101.3, t | 5.87, s | 101.2, t | 5.83, s |
| 1′ | 138.2, s | | 138.3, s | |
| 2′ | 106.6, d | 6.11, d, $J = 1.9$ Hz | 106.1, d | 6.15, d, $J = 1.8$ Hz |
| 3′ | 152.2, s | | 152.5, s | |
| 4′ | 140.2, s | | 140.0, s | |
| 5′ | 152.2, s | | 152.5, s | |
| 6′ | 106.6, d | 6.11, d, $J = 1.9$ Hz | 106.1, d | 6.15, d, $J = 1.8$ Hz |
| 7′ | 10.2, q | 0.94, d, $J = 7.1$ Hz | 20.3, q | 0.99, d, $J = 6.8$ Hz |
| 8′ | 36.6, d | 2.28–2.34, m | 28.5, d | 2.31–2.34, m |
| 9′α | 66.8, t | 3.79, d, $J = 13.6$ Hz | 20.0, q | 1.02, d, $J = 6.8$ Hz |
| 9′β | | 3.93, dd, $J = 11.2$, 6.6 Hz | | |
| OMe-5 | 55.9, q | 3.73, s | 56.0, q | 3.83, s |
| OMe-3′ | 55.7, q | 3.70, s | 55.8, q | 3.79, s |
| OMe-4′ | 60.7, q | 3.72, s | 60.8, q | 3.81, s |
| OMe-5′ | 55.7, q | 3.70, s | 55.8, q | 3.79, s |
| OH-9′ | | 5.01 br s | | |

suggested the existence of a OH–$CH_2$–CH($CH_3$)–CH($CH_3$)–CH structural unit in **1**. The ^{1}H and ^{13}C NMR spectra of **1** are similar to those of 4,4-di(4-hydroxy-3-methoxyphenyl)-2,3-dimethylbutanol [18]. The obvious differences are the chemical shift of aromatic rings, and the substituent groups on aromatic rings. The HMBC correlations of H-10 ($\delta_H$ 5.87, s) with C-3 ($\delta_C$ 148.5) and C-4 ($\delta_C$ 133.9) indicated that the methylenedioxy group should be located at C-3 and C-4. The HMBC correlations of four methoxyl groups with C-5, C-3′, C-4′, and C-5′ indicated that four methoxyl groups should be located at C-5, C-3′, C-4′, and C-5′, respectively. As the C—C bonds can rotate randomly, the relative configuration of compound **1** could not be determined on the basis of ROESY spectrum. Thus, the structure of compound **1** was determined as shown in Figure 1, and this compound is named as schilancifolignan D.

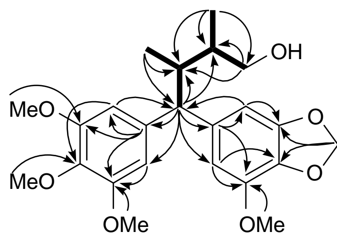


Figure 2. Selected HMBC (→) and ^{1}H–^{1}H COSY (—) correlations of **1**.

Compound **2** was also obtained as a yellow gum. It was assigned the molecular formula $C_{23}H_{30}O_6$ by its HR-ESI-MS at $m/z$ 425.1932 $[M + Na]^+$. Its ^{1}H and ^{13}C NMR spectra showed signals of 30 protons and 23 carbons, respectively, corresponding to two aromatic rings with four aromatic protons, three methyl groups,

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four methoxyl groups, one methylenedioxy group, and three methine signals. The $^1$H and $^{13}$C NMR spectra of **2** are similar to those of compound **1**. Analysis of the $^1$H and $^{13}$C NMR spectral data of **2** with those of **1** suggested that the difference was that an oxidated methylene group (C-9) in **1** was substituted by a methyl group in **2**, which was supported by the disappearance of the signal of an oxidated methylene group and the appearance of a methyl group at $\delta$ 1.23 (d, $J = 6.7$ Hz) in **2**. Thus, the planar structure of compound **2** was determined as shown in Figure 1, and this compound was named as schilancifolignan E.

## 3. Experimental

### 3.1 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 spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometer with TMS as internal standard. Unless otherwise specified, chemical shifts ($\delta$) were expressed in ppm with reference to the solvent signals. HR-ESI-MS 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 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, and spots were visualized by heating silica gel plates sprayed with 5% $H_2SO_4$ in EtOH.

### 3.2 Plant material

The leaves and stems of *S. lancifolia* were collected in Erlang Mountain area of Sichuan Province, China, in September 2007. The identification of plant material was verified by Prof. Xi-Wen Li. A voucher specimen (KIB-07-09-28) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China.

### 3.3 Extraction and isolation

The air-dried and powdered leaves and stems of *S. lancifolia* (3.2 kg) were extracted four times with 70% aqueous $Me_2CO$ (4 × 3.5 liters) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure and partitioned with EtOAc (3 × 4 liters). The EtOAc partition (158 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, and 5:5) to give six fractions A–F. The separation of fraction B (9:1, 18.9 g) by silica gel column chromatography eluted with petroleum ether–acetone (9:1–1:2) yielded mixtures B1–B6. Fraction B2 (8:2, 3.25 g) was subjected to silica gel column chromatography using petroleum ether–acetone (8:2) and preparative HPLC (68% MeOH–$H_2O$, flow rate 12 ml/min) to give compounds **2** (5.4 mg), **5** (25.2 mg), **8** (18.5 mg), and **9** (35.2 mg). Fraction B3 (7:3, 1.86 g) was subjected to silica gel column chromatography eluting with petroleum ether–acetone (7:3) and then run on preparative HPLC (63% MeOH–$H_2O$, flow rate 12 ml/min) to yield compounds **1** (4.2 mg), **4** (26.2 mg), and **7** (19.5 mg). Fraction B4 (6:4, 1.24 g) was subjected to silica gel column chromatography eluting with petroleum ether–acetone (6:4) and then run on preparative HPLC (58% MeOH–$H_2O$, flow rate 12 ml/min) to give compounds **3** (6.8 mg), **6** (13.4 mg), and **10** (11.6 mg).

Table 2. Cytotoxicities of schilancifolignans D and E.

| Compounds | Cell lines | | | |
|---|---|---|---|---|
| | HL-60 | HepG2 | KB | MDA-MB-231 |
| **1** | 4.15 | 12.2 | 8.21 | 18.4 |
| **2** | 5.21 | 7.26 | 12.4 | 8.57 |
| Camptothecin | 1.18 | 0.62 | 1.26 | 1.87 |

Notes: Data are $IC_{50}$ values in μmol/l. For a compound to be deemed effective, an $IC_{50}$ value < 100 μmol/l is required. Camptothecin was used as a positive control. HL-60, human acute promyelocytic leukemia; Hep-G2, human hepatocellular carcinoma; KB, human oropharyngeal epidermoid carcinoma; MDA-MB-231, human breast cancer cells.

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### 3.4 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 ($EC_{50}$) [19]. Schilancifolignan D shows anti-HIV-1 activity with $EC_{50}$ 3.62 μg/ml, $CC_{50}$ 56.6 μg/ml, and therapeutic index (TI) 15.6. Schilancifolignan E shows anti-HIV-1 activity with $EC_{50}$ 2.87 μg/ml, $CC_{50}$ 51.4 μg/ml, and TI 17.9.

### 3.5 Cytotoxicity assays

The cytotoxicity tests for the isolates were performed using a previously reported procedure [20]. All treatments were performed in triplicate. In the MTT assay, $IC_{50}$ was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic ability against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control) was shown in Table 2.

#### 3.5.1 Schilancifolignan D (1)

Obtained as a pale yellow amorphous solid; $[\alpha]_D^{15.8}$ + 8.4 ($c = 0.18$, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 326 (2.58), 282 (4.15), 205 (4.95) nm; IR (KBr) $\nu_{max}$ 3340, 2962, 2932, 2875, 2834, 1610, 1595, 1518, 1460, 1415, 1378, 1330, 1267, 1240, 1228, 1185, 1143, 1026, 972, and 823 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data (Table 1); positive ESI-MS $m/z$ 441 $[M + Na]^+$; HR-ESI-MS: $m/z$ 441.1895 $[M + Na]^+$ (calcd for $C_{23}H_{30}O_7Na$, 441.1889).

#### 3.5.2 Schilancifolignan E (2)

Obtained as a yellow gum; $[\alpha]_D^{21.9}$ + 9.7 ($c = 0.15$, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 326 (2.65), 282 (4.11), 205 (5.01) nm; IR (KBr) $\nu_{max}$ 2974, 2945, 2880, 2842, 1618, 1595, 1521, 1460, 1426, 1375, 1257, 1264, 1148, 1022, 976, and 825 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data (Table 1); positive ESI-MS $m/z$ 425 $[M + Na]^+$; HR-ESI-MS (positive ion mode): $m/z$ 425.1932 $[M + Na]^+$ (calcd for $C_{23}H_{30}O_6Na$, 425.1940).

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